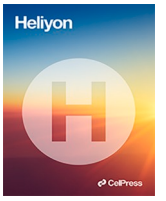
Heliyon 10 (2024) e39701

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Sida corymbosa prevents seizures and memory deficit via inhibition of oxidative stress and neuronal loss in the hippocampus

Marguerite Zizanie Penda [a], Antoine Kavaye Kandeda [a,*], Frida Longo [b], Livana Brinda Ateufack [a], Théophile Dimo [a]

[a] *Department of Animal Biology and Physiology, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon*
[b] *Department of Biological Sciences, Higher Teacher Training College, University of Yaoundé I, P.O. Box 3805, Yaoundé, Cameroon*

A R T I C L E I N F O

Keywords:
γ-Aminobutyric acid
Acetylcholine
Antiepileptic
Anti-amnesic
Morris water maze
Barnes maze
Novel object recognition
Pentylentetrazole kindling model
Oxidative stress
Sida corymbosa

A B S T R A C T

Mesiotemporal lobe epilepsy is the most refractory form of epilepsy. The management of this condition relies on the use of antiepileptic drugs known to provide symptomatic relief. Herbal remedies are therefore an alternative to finding a cure for epilepsy. The present study aimed to assess the antiepileptic and anti-amnesic effects of *Sida corymbosa* aqueous extract using the pentylenetetrazole (PTZ) kindling model in rats. For this, 48 rats were divided into 6 groups of 8 animals each and treated as follows: normal and negative controls received *per os* (p.o) distilled water (10 mL/kg); experimental groups received *S. corymbosa* extract (17.14, 34.28, and 68.56 mg/kg, p.o); and positive control group received levetiracetam (250 mg/kg, p.o). One hour after the treatments, animals intraperitoneally (i.p) received a subconvulsive dose of PTZ (25 mg/kg), except the normal control group. The animals were treated once daily until the onset of the first tonic-clonic seizure (stage 5) in the negative control group. Twenty-four hours following the onset of the first seizure, memory impairment was assessed. Twenty-four hours later, the animals were sacrificed, and the hippocampus and prefrontal cortex were collected for biochemical analyses. The extract (68.56 mg/kg) prevented ($p < 0.001$) seizures from day 1 to day 12 compared to the negative control group. The extract (17.14 and 34.28 mg/kg) increased the recognition index by 80 % ($p < 0.05$) and 60 % ($p < 0.01$), respectively, compared with the negative control group. Moreover, the extract (17.14 mg/kg) increased by 68 % ($p < 0.001$) the concentration of GABA in the hippocampus. The extract (17.14 and 34.28 mg/kg) increased the acetylcholine level by 51 % ($p < 0.001$) and 69 % ($p < 0.001$) in the hippocampus, respectively. The extract at a dose of 17.14 mg/kg decreased the concentration of MDA in the hippocampus by 43 % ($p < 0.001$). These findings suggest that the extract has antiepileptic and anti-amnesic potentials that could justify its empirical use.

1. Introduction

Epilepsy is a chronic neurological disease characterized by excessive electrical discharges from a group of neurons in the brain [1].

* Corresponding author.
E-mail addresses: radiependa@gmail.com (M.Z. Penda), kandedakavaye@yahoo.fr, antoine.kandeda@facsciences-uy1.cm (A.K. Kandeda), longofrida@yahoo.fr (F. Longo), ateufacklivane@gmail.com (L.B. Ateufack), dimo59@yahoo.com (T. Dimo).

https://doi.org/10.1016/j.heliyon.2024.e39701
Received 23 May 2024; Received in revised form 16 October 2024; Accepted 21 October 2024
Available online 22 October 2024

It affects around 70 million people worldwide, 80 % of whom live in developing countries [1,2]. Seizures are clinical manifestations of hypersynchronous and self-sustained paroxysmal discharge of a more or less large population of neurons [3]. Risk factors of epilepsy include stroke, infections, brain tumors, prolonged fever, and head trauma [4]. The hippocampus is one of the brain regions most frequently affected by epileptic seizures. This form of epilepsy is known as mesiotemporal lobe epilepsy (MTLE). This condition is characterized by recurrent, unprovoked focal seizures in the temporal lobe, and is often associated with hippocampal sclerosis or atrophy [4]. It is estimated that around 16 ‰ people suffer from this form of epilepsy in Cameroon [5]. Moreover, MTLE is generally associated with several neuropsychiatric comorbidities such as cognitive impairment, anxiety, and depression [6,7]. Despite the use of old antiepileptic drugs (valproate sodium, diazepam, phenobarbital) and the development of new drugs (levitiracetam, oxcarbazepine, carbamazepine, clobazam, vigabatrin), approximately one-third of patients with MTLE remain seizure-prone [4]. Indeed, current antiepileptic drugs only target symptoms rather than the underlying causes of the disease and associated comorbidities [7,8]. There is therefore a great interest in developing new drugs that effectively target the underlying causes of the disease.

It has been established that during MTLE, spontaneous recurrent seizures are correlated with a decrease in γ-Aminobutyric acid (GABA) level, an increase in GABA-transaminase (GABA-T) activity, and a decrease in L-glutamate decarboxylase activity in hippocampal and prefrontal cortex tissues [9,10]. In addition to the imbalance between GABAergic and glutamatergic neurotransmissions, the pathogenesis of MTLE involves oxidative stress and the generation of free radicals [11]. The generation of reactive oxygen species and free radicals is thought to be one of the mechanisms by which recurrent seizures occur in the brain [11,12]. Although there is no experimental model that can mimic all the features of MTLE, the pentylenetetrazole (PTZ) kindling model is a widely accepted animal model to assess new antiepileptic drugs or to study the pathogenesis of epilepsy, compared with other models such as pilocarpine, 4-aminopyridine, and kainate models of epilepsy [13,14]. Kindling is the repeated administration of subconvulsive electrical or chemical stimulation that lowers the epileptogenic threshold until the onset of generalized tonic-clonic seizures [7,15]. In addition, kindling causes the development of partial seizures that lead to generalized tonic-clonic seizures and associated cognitive impairment [15,16]. Kindling seizures can cause loss of neurons (hippocampal sclerosis) in the CA1, CA3, and dentate gyrus regions of the hippocampus and surrounding areas (amygdala, striatum, subiculum, cortex entorhinal) [7,17]. An accumulating body of research has shown that increased glutamatergic transmission plays a pivotal role in the generation of free radicals and pro-inflammatory cytokines in the brain [18,19]. Besides, recurrent seizures in MTLE are strongly associated with severe cognitive impairment [20–22]. Thus, the current understanding of epilepsy suggests that the discovery of antiepileptic drugs with antioxidant and anti-inflammatory activities is beneficial, and medicinal plants are a prime alternative to solve this problem. Cameroon flora abounds in plants that are used empirically to treat some neurological conditions such as epilepsy, Alzheimer's disease, neuropathy, headaches, autism, and Parkinson's disease.

Sida corymbosa R.E. Fries is a basal, upright, and perennial shrub that can reach a meter in height. Its taxonomy is as follows: Equisetopsida (Class); Magnoliidae (Subclass); Malvales (Order); Rosanae (Suborder); Malvaceae (Family); Sida (Genus); *Sida corymbosa* (Species) [23]. This plant is used in traditional medicine to treat migraine, urinary infections, malaria, diarrhea, neurological disorders, inflammations, gastric ulcers, and epilepsy in most African (Cameroon and Nigeria) and Asia countries [23]. *Sida corymbosa* possesses anti-hemorrhagic, wound healing, anti-ulcer, and antibacterial properties [23–25]. This medicinal plant has been shown to regulate abnormal serum bilirubin, cardiac irregularity, liver functions, fever, seizures, rheumatism, painful micturition, and to prevent general debility and mental illness in rats [23–25]. Acute toxicity studies of the leaf extract exceeded 5000 mg/kg body weight in rats [25]. Phytochemical screening of the leaf extract revealed the presence of saponins, tannins, phenols, terpenoids, flavonoids, and alkaloids [25], while quantitative phytochemical analysis indicated the presence of spartein, epicatechin, anthocyanin, tannin, phytate, phenol, lunamarine, sapogenin, naringin, catechin, rutin, ribalinidine, and kaempferol [26]. According to several studies, the presence of these bioactive compounds may partly explain the antioxidant and anti-inflammatory properties of the extract [25]. To date, no scientific studies have been carried out on the antiepileptic and anti-amnestic properties of this plant. Furthermore, given the antioxidant and anti-inflammatory potentials of the aqueous leaf extract, the present study aimed to investigate the antiepileptic and anti-amnesic effects of the aqueous leaf extract of *Sida corymbosa* leaves using the PTZ-kindling model in rats. The effect of the extract on the concentration of some neurotransmitters (γ-Aminobutyric acid and acetylcholine), oxidative status parameters (malondialdehyde and reduced glutathione), and neuronal loss in the CA (cornu ammonis)1, CA2, CA3, and dentate gyrus regions were also explored.

2. Materials and methods

2.1. Chemicals, reagents, and drugs

Pentylenetetrazole (PTZ), levetiracetam, thiobarbituric acid, trichloroacetic acid, sodium bicarbonate, sodium carbonate, ketamine, pentobarbital, copper sulfate, monosodium phosphate monobasic and dibasic, sodium potassium tartrate, glutamate, hydrochloric acid, Ellman's reagent, iron (III) chloride, sodium chloride, hydroxylamine, physostigmine, γ-Aminobutyric acid, 5,5′-dithio bis-2-nitrobenzoic acid, ninhydrin, pyridoxal-5-phosphate, butylated hydroxytoluene, hematoxilin, eosin, phosphate buffer, tris base, Folin and Ciocalteu reagent, rutin, methanol, aluminum chloride, sodium acetate, gallic acid, vanillin, catechin, n-butanol, ammonium hydroxide, and formaldehyde were purchased from Sigma Chemical Lab. (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Plant material and extract preparation

Sida corymbosa leaves (Fig. 1) were selected following several ethnobotanical surveys. The leaves of *S. corymbosa* were harvested in

the early hours of the morning, in the locality of Nomayos, Mefou-et-Akono division (Centre-Cameroon region), in September 2021. The plant was identified at the National Herbarium of Cameroon by comparison with Swadick J.T (botanist who first discovered the species), sample N°SCA 262, registered under number 33892 / HNC.

The preparation method was that of a traditional healer (50 fresh leaves in 1 L of tap water). The traditional healer picks 50 fresh leaves, weighing between 80 and 100 g, and macerates them in 1L of tap water for 24 h (personal communication). This procedure was mimicked in our laboratory. Thus, 50 fresh leaves of *Sida corymbosa* were collected, washed with tap water, dried in the shade, and reduced to powder. Fifty grams of the powder were macerated in 5 L of distilled water for 24 h. After maceration, the mixture was filtered using the Whatman paper No. 3, and the obtained filtrate was oven-dried at 45 °C. The doses of *Sida corymbosa* aqueous extract tested in this study were calculated based on information gathered from the traditional healer. Indeed, after preparing his macerates, the traditional healer gave his patient 1L per day for two weeks. This content was then evaporated in an oven to obtain a dry extract (2.4 g). This mass represented the amount of dry extract taken by a man weighing around 70 kg. This information was used to determine the equivalent human dose, estimated at 34.28 mg/kg. This dose was framed by a lower dose (17.14 mg/kg) obtained by dividing the stock solution by a factor of 2, and a higher dose (68.56 mg/kg) by multiplying the stock solution by a factor of 2.

2.3. Animal housing conditions and ethical consideration

Male rats of the Wistar strain, approximately three months old, and weighing an average of 200 g, were used in this study. The study was carried out at the Laboratory of Animal Physiology, of the Faculty of Science, University of Yaoundé I. The animals were kept in plastic cages, under conditions of ambient temperature (24–26°C) and adequate ventilation. During the experiment, they had free access to water (tap water) and food. For 50 kg bags, their food consisted of 60 % corn, 20 % smoked fish, 10 % wheat, 5 % peanuts, 4 % bone powder, and 1 % multivitamin complex [27]. All studies were carried out following the Cameroon National Ethics (N° FWA-IRB00001954) governing the use of laboratory animals.

2.4. Experimental design and PTZ-kindling

Kindling is the repeated administration of subconvulsive electrical or chemical stimulation that lowers the seizure threshold until the onset of generalized tonic-clonic seizures [7,15]. It causes simple partial seizures to evolve into generalized tonic-clonic seizures [24]. On the first day of the experiments, rats were marked, weighed, and divided into six groups of eight animals each, as follows (Table 1): the normal control group (named DW + DW group) was only treated with distilled water (10 mL/kg; *per os*); the negative control group (named DW + PTZ group) treated with distilled water (10 mL/kg; *p.o.*); three test groups (named SC 17.14 + PTZ; SC 34.28 + PTZ; and SC 68.56 + PTZ groups) were treated with the extract of *S. corymbosa* (17.14, 34.28, and 68.56 mg/kg; respectively, *p.o.*); and the positive control group (named Lev + PTZ group) treated with levetiracetam (250 mg/kg; *p.o.*). One hour following the administration of the above treatments, a subconvulsive dose of PTZ (25 mg/kg) was intraperitoneally (i.p) injected into all rats, with the exception of those in the normal control group treated instead with distilled water (10 mL/kg; *i.p.)*. These co-administrations (PTZ and various treatments) were repeated once a day, until the onset of stage 5 seizures, on day 20 in the negative control group [28]. During this period, each animal was individually observed for 30 min to detect the onset of seizures. The number and duration of any seizures were recorded using the Racine scale [29]: stage 1 (hyperactivity, twitching, and/or convulsions); stage 2 (head shaking, nodding, and myoclonic convulsions); stage 3 (unilateral forelimb convulsions); stage 4 (recovery with bilateral forelimb convulsions); and stage 5 (generalized tonic-clonic convulsions with loss righting reflex). Twenty-four hours after the onset of the first seizure (stage

Fig. 1. The whole part of *Sida corymbosa.*

Table 1
Distribution and treatment of rats.

Groups	Nb rats	Treatment phase (1st administration)	Latency	Induction phase (2nd administration)	Co-administration (1st + 2 nd)	Abbreviation
Normal control group	8	Distilled water (10 mL/kg, p.o.)	1 h	Distilled water (10 mL/kg, i.p)	Once daily for 20 days	DW + DW
Negative control group	8	Distilled water (10 mL/kg, p.o.)	1 h	Pentylenetetrazole (25 mg/kg, i.p)	Once daily for 20 days	DW + PTZ
Test groups	8	*S. corymbosa* (17.14 mg/kg, p.o.)	1 h	Pentylenetetrazole (25 mg/kg, i.p)	Once daily for 20 days	S. C17.14+PTZ
	8	*S. corymbosa* (34.28 mg/kg, p.o.)	1 h	Pentylenetetrazole (25 mg/kg, i.p)	Once daily for 20 days	S. C34.28+PTZ
	8	*S. corymbosa* (68.56 mg/kg, p.o.)	1 h	Pentylenetetrazole (25 mg/kg, i.p)	Once daily for 20 days	S. C68.56+PTZ
Positive control group	8	Levetiracetam (250 mg/kg, p.o.)	1 h	Pentylenetetrazole (25 mg/kg, i.p)	Once daily for 20 days	Lev + PTZ

Nb = Number; p.o. = *per os*; i.p = Intraperitoneally; Lev = Levetiracetam; S.C = *Sida corymbosa*; PTZ = Pentylenetetrazole; DW = Distilled water; 1st = Fist administration; 2nd = Second administration.

5) was reached in the negative control group, on day 22, the object recognition test was performed for three days. Twenty-four hours later, on day 26, the Morris water maze test was performed for 6 days. Finally, on day 33, the Barnes maze test was performed for 5 days. During the behavioral assessment, treatments were continued until the end of the experiment. At the end of behavioral studies, the rats were euthanized, and the prefrontal cortex and hippocampus were collected for biochemical and histological analysis.

2.5. Behavioral assessment

2.5.1. Novel object recognition task

This test was performed on days 22, 23, and 24 and aimed to assess recognition memory in rodents. It was carried out according to a modified protocol [30]. The animal was placed in an open arena measuring 50 × 50 × 20 cm. The first stage (habituation phase) of the test began with the acclimatization or habituation of the animal with a device (painted in white) for 5 min. The second stage (acquisition phase) took place 24 h after the acclimatization or habituation phase. During this stage, the animal was allowed to freely explore two identical objects (considered familiar objects) for 5 min. After this time, the animal was returned to its cages. Twenty-four hours later, the third stage (retention phase) was performed. During this step, the white color of the device was changed to red, and one of the two identical objects was replaced by a new one (novel object). The time to explore (recognition memory) each object (familiar or novel) was evaluated for 5 min. This time was subsequently converted into recognition index [31]:

$$\boldsymbol{RI} = \frac{TN}{(\boldsymbol{T_{N+}}\ \boldsymbol{T_F})} \times 100$$

Where: T_N: time to explore novel object; T_F: time to explore familiar object; RI: recognition index.

2.5.2. Morris water maze task

Spatial learning and long-term memory impairments were assessed using the Morris water maze task [32]. A circular pool of 150 cm in diameter and 60 cm in height, filled to 40 cm with water at 25 °C, was divided into four equal quadrants with a white refuge platform of 8 cm in diameter and 30 cm in height. This platform was placed in the center of one of the quadrants, 1 cm below the water surface. The pool was located in a room with multiple visual cues. On the first day of testing, i.e. during the habituation phase, each rat was acclimatized for 60 s without the platform. Each rat performed sessions of three-block with 30-min intervals between sessions. Each block consisted of four successive trials lasting 60 s. In each trial, rats were randomly released into the water from one of the four quadrants facing the maze wall [32]. The acquisition phase began on day 2 with the refuge platform and continued for 4 days with three sessions per day. The water was made cloudy by adding liquid milk so that the platform was invisible on the water surface. The session time for each animal to find the platform was 120 s, and the time interval between sessions was 5 min. Once the animal found the platform, it was allowed to remain there for 15 s. During each session of the acquisition phase, the latency to find the platform was recorded for each animal. The effectiveness of learning efficiency was then assessed in the retention phase on day 6. During this phase which, lasted 60 s, the platform was removed from the tank and the time spent in this quadrant was recorded [32].

2.5.3. Barnes maze task

Barnes maze is a device used in psychological laboratory experiments to evaluate long-term memory and spatial learning [33]. It was first developed by Barnes [34], and it consisted of a circular surface with 20 circular holes around its circumference. Visual cues, such as shapes or colorful patterns are placed around a table within sight of the animal. Under one of the holes, there was an "escape box" that the animal could reach [35]. The test took place over five days. The animals were placed in the center of the device, and each animal was given a 120-s trial period to find the escape box. The timer was directly stopped when the animal entered the hole containing the escape box, and the animal was allowed to remain there for 15 s. The device was then cleaned with 70 % ethanol before the

next animal [35]. Animals that did not find the hole of interest within this timeframe were directed to it and remained there for 15 s. The aim was to guide and enable the animal to remember the position of the target (hole) using the surrounding signals. This phase (acquisition phase) took place over 4 days, with 4 sessions per animal at different starting positions. During each session of the acquisition phase, the latency to find the target hole was recorded for each animal. On the fifth day (test day or retention phase), the animals were placed in the center of the device and held for 60 s. The following parameters were recorded during this phase: the number of errors and the time spent by the animal to find the hole of interest [35].

2.6. Sacrifice and preparation of homogenates

Immediately after the Barnes maze task, all animals were sacrificed by cervical dislocation under anesthesia with ketamine (50 mg/kg, i.p) and pentobarbital (30 mg/kg, i.p.). The brains were removed, washed in 0.9 % NaCl (9 g of NaCl dissolved in 1 L of distilled water), blotted, weighed, and placed in beakers containing frozen 0.9 % NaCl for solidification. After solidification, the organs were dissected to remove the prefrontal cortex and hippocampus. The hippocampi and prefrontal cortex (n = 5) were homogenized in a ceramic mortar containing 50 mM Tris-HCl buffer (pH 7.4; 10 mL of 0.5 M Tris base mixed with approximately 80 mL of water, and the pH adjusted to 7.4 with HCl. This was then diluted to 100 mL with distilled water) to prepare 10 % homogenate (1 g of brain tissue homogenized in 10 mL of 50 mM of Tris-HCl buffer). The resulting mixture was then centrifuged at 1.742×*g* for 15 min using an MF-80–1D centrifuge (Wincom Company Ltd. Changsha, Hunan, China). The supernatant was collected and used for neurochemical analyses. In addition, the remaining hippocampi (n = 3) were fixed in 10 % formalin (1 part of formalin stock with 9 parts distilled water. The solution was then adjusted to neutral pH with 4 g/L monosodium phosphate monobasic and 6.5 g/L monosodium phosphate dibasic/anhydrous). The histological sections were realized in the hippocampus because this structure is mainly involved in seizure generation, i.e. seizure foci in temporal lobe epilepsy [36,37].

2.7. Biochemical analyses

2.7.1. Determination of γ-Aminobutyric acid (GABA) level

γ-Aminobutyric acid level in the brain was assessed using the method described by Lowe [38]. It is based on the coloration generated by the reaction of GABA and ninhydrin in an alkaline medium, in the presence of glutamate. The reaction medium consisted of 200 μL of 0.14 M ninhydrin prepared in carbonate-bicarbonate buffer (0.5 M, pH 9.9; 1.05 g of sodium bicarbonate and 9.27 g of sodium carbonate dissolved in 1000 mL of distilled water) and 100 μL of 10 % glacial trichloroacetic acid (TCA), 100 μL of homogenate (1 g of brain tissue homogenized in 10 mL of 50 mM of Tris-HCl buffer) was added and the whole was incubated at 60 °C for 30 min. After cooling, the entire contents of each tube were added to tubes containing 5000 μL of copper tartrate (0.1 g of copper sulfate and 0.2 g of sodium potassium tartrate each dissolved in approximately 10 mL of deionized water. Both solutions were added to 0.5 g/mL of sodium carbonate and brought to a volume of 100 mL). The tubes were then incubated at 25 °C for 10 min. The absorbance of the mixture was read by a spectrophotometer (Yoke Instrument Co. Ltd, Shangai, China) at 451 nm against the blank and was proportional to GABA concentration in the sample. The determination of this concentration was made from the calibration curve of the standard GABA solution, obtained by dissolving GABA (50, 100, 150, 200, 250, 300, 350 et 400 μg) mixed with 0.5 mg of L-glutamate in 2 mL of 10 % TCA. The concentration of GABA in the homogenate was expressed as μg/g of wet tissue.

2.7.2. Determination of L-glutamate decarboxylase (L-GAD) activity

The activity of L-GAD was determined by the colorimetric method of Nayak [39]. In the presence of L-GAD, L-glutamic acid is metabolized to GABA, which absorbs at 455 nm. In dry tubes, 1 mL of homogenate and 1 mL of L-glutamic acid (0.05 M; pH 6.7) were added, followed by 0.1 mL of pyridoxal-5-phosphate (50 mM). The mixture was incubated at 37 °C for 30 min, then at 60 °C for 10 min. The absorbance of GABA in the mixture was read by a spectrophotometer at 455 nm, at 30 s and 90 s relative to the blank. The activity of L-glutamate decarboxylase (L-GAD) was reported as *p*g of GABA/min/mg of fresh tissue. To calculate the L-GAD activity in the homogenate, the Beer-Lambert law was applied.

$$Activity\ \ of\ \mathbf{L}-GAD\ (pg\ de\ GABA\ /\ \mathbf{min}\ /\ \mathbf{g}) = \frac{\Delta DO \times Vt \times \mathbf{10^6}}{\mathbf{m} \times \boldsymbol{\varepsilon} \times \mathbf{l} \times \mathbf{t} \times Ve}$$

Where: ΔDO: variation of optical densities; Vt: total volume of reaction medium (ml); m: mass of tissue used (g); ε: molar extinction coefficient = 40 M-1.cm-1; Ve: volume of homogenate used for analyses (ml); l: optical path of cell = 1 cm; t: time (min).

2.7.3. Determination of acetylcholine level

In the presence of acetylcholinesterase, acetylcholine is broken down into thiocholine. The reaction of this metabolite with the thiol group of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) leads to the generation of a yellow complex called thionitrobenzoic [40]. The absorbance of the latter at 540 nm is proportional to the concentration of acetylcholine in the medium. To 0.8 mL of homogenate in dry tubes, 1.4 mL of distilled water, 0.2 mL of physostigmine (1.5 mM), and 0.8 mL of TCA (1.84 M) were subsequently added. The mixture was centrifuged and 1 mL of the supernatant was collected in dry tubes. Then, 1 mL of basic hydroxylamine, 10.5 mL of HCl (4 M), and 0.5 mL of $FeCl_3$ (0.37 M) were added to these tubes. The resulting mixture was incubated at 25 °C for 15 min, and the absorbance was read at 540 nm by a spectrophotometer [39]. Acetylcholine level in the tissue was reported as μmol/g of wet tissue [40]. To determine the concentration of acetylcholine in the homogenate, the Beer-Lambert law was used [40].

$$Concentration\ of\ acetylcholine\ (\mu mol/\mathbf{g}) = \frac{OD \times Vt \times \mathbf{10^6}}{\mathbf{m} \times \boldsymbol{\varepsilon} \times \mathbf{l} \times Ve}$$

Where: OD: optical density; Vt: total volume of reaction medium (ml); m: mass of tissue used (g); ε: molar extinction coefficient = 40 M-1.cm-1; Ve: volume of homogenate used for analyses (ml); l: optical path of cell = 1 cm.

2.7.4. Determination of malondialdehyde (MDA) level

Two hundred and 50 μL of homogenate were introduced into test tubes. Then, 250 μL of 20 % trichloroacetic acid (TCA), 500 μL of 0.67 % thiobarbituric acid (TBA), and 10 μL of 0.1 % butylated hydroxytoluene (BHT) were added to each tube. The blank solution consisted of all the above-stated items except the homogenate. The tubes were sealed, incubated at 90 °C for 10 min, and cooled with tap water. The result was centrifuged at 1.742×*g* for 10 min at room temperature. The supernatant was collected and the absorbance read at 532 nm relative to the blank. The concentration of MDA was expressed as nmol/mg of wet tissue [41]. To calculate the concentration of MDA in the supernatant, the Beer-Lambert law was used [41].

$$Concentration\ de\ MDA\ (nmol/mg) = \frac{OD \times Vt}{\mathbf{m} \times \boldsymbol{\varepsilon} \times \mathbf{l} \times Ve}$$

Where: OD: optical density; Vt: total volume of reaction medium (ml); m: mass of tissue used (g); ε: molar extinction coefficient = 40 M-1.cm-1; Ve: volume of homogenate used for analyses (ml); l: optical path of cell = 1 cm.

2.7.5. Determination of reduced glutathione (GSH) level

A volume of 1500 μL of the Ellman's reagent (DTNB, 0.1 mM 5,5′-dithio bis-2-nitrobenzoic acid in 0.3 M phosphate buffer with 1 % of sodium citrate solution) was introduced into tubes previously containing 100 μL of homogenate (test tube) and 100 μL of phosphate buffer. The mixture was incubated for 1 h at room temperature and the absorbance was read by a spectrophotometer at 412 nm relative to the control. The control solution consisted of all the above-stated items except the homogenate. The concentration of GSH in the homogenate was determined according to Beer-Lambert law and expressed as nmol/mg of wet tissue [42].

$$Concentration\ de\ GSH\ (nmol/\mathbf{g}) = \frac{OD \times Vt}{\mathbf{m} \times \boldsymbol{\varepsilon} \times \mathbf{l} \times Ve}$$

Where: OD: optical density; Vt: total volume of reaction medium (ml); m: mass of tissue used (g); ε: molar extinction coefficient = 40 M-1.cm-1; Ve: volume of homogenate used for analyses (ml); l: optical path of cell = 1 cm.

2.7.6. Histopathological analysis of brain tissues

The histological analysis included fixing, cutting, dehydration, inclusion, cutting, coloring, mounting, and observation [43]. The stained (hematoxylin and eosin) and mounted slides were observed with Scientico STM-50 optical microscope (HSIDC Industrial Estate, Haryana, India) equipped with a Celestron 44421 digital camera connected to an HP pavilion g6-100 notebook computer (Hewlett-Packard Inc. Chongqing, China) using ImageJ software version 1.520 [43]. The obtained images were also used for cell counting in the CA1, CA2, CA3, and dentate gyrus regions of the hippocampus [43].

2.8. Quantitative phytochemical analysis

The concentrations of total phenolic compounds, total flavonoids, condensed tannins, total alkaloids, and saponins were performed in triplicate.

2.8.1. Total phenolic compounds estimation

Total phenolic compounds was determined using the method of Folin and Ciocalteu (FC) [44]. Briefly, 100 μL of *S. corymbosa* extract (0.02–0.15 mg/mL) or 100 μL of gallic acid (0.02–0.15 mg/mL) was mixed with 400 μL of 7.5 % sodium carbonate and 500 μL of FC reagent. The mixture was incubated for 10 min at room temperature and the absorbance was read at 730 nm by a spectrophotometer. Total phenol content in gallic acid equivalent was calculated using the gallic acid calibration curve. This concentration was expressed as mg gallic acid equivalent (GAE)/g of extract.

2.8.2. Total flavonoids concentration

The determination of total flavonoid concentration was performed according to Dehpour method [45]. Succinctly, 500 μL *S. corymbosa* extract (0.02–0.15 mg/mL) or 500 μL rutin (0.02–0.15 mg/mL) were added to 1500 μl of 95 % (v/v) methanol, 100 μL of 10 % (m/v) aluminum chloride, 100 μL of 1M sodium acetate, and 2.8 mL of distilled water. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was determined at 415 nm by a spectrophotometer. The concentration of total flavonoids in the extract was obtained from the rutin calibration curve. The concentration of total flavonoid in the extract was expressed as mg rutin equivalent (RE)/g of extract.

2.8.3. Condensed tannins

The concentration of tannins was estimated by the method described by Mang'Dobara et al. [46]. Equal volumes of 37 % methanol

(v/v), 4 % vanillin in methanol (m/v), and 8 % hydrochloric acid (v/v) were mixed. The mixture was stored at 30 °C before the assay. Two hundred microliters of *S. corymbosa* extract (0.06–0.3 mg/mL) or catechin (0.06–0.3 mg/mL) were then added to 1000 μL of the above-mentioned mixture. The obtained solution was incubated at 30 °C for 20 min in the dark. The optical density was determined at 500 nm by a spectrophotometer. The condensed tannins were estimated from the calibration curve of catechin and expressed as mg catechin equivalent (Cat E)/g of extract [47].

2.8.4. Total alkaloids concentration

Six grams of *S. corymbosa* extract was dissolved in 200 mL of 10 % acetic acid in ethanol (v/v). The solution was covered, allowed to stand for 4 h, and filtered. The resulting solution (initial solution) was concentrated in a water bath to 1/4 of the initial volume. Ammonium hydroxide was then added in a drop-wise manner until the precipitation was complete in the solution. The precipitate was collected, washed with diluted ammonium hydroxide, and finally filtered. The residue (alkaloids) was dried and weighed [48]. The concentration of total alkaloids in the extract, expressed as % total alkaloids, was calculated as follows [48]:

% total alkaloids = [mass of alkaloids (mg)/mass of *S. corymbosa* extract (g)] * 100.

2.8.5. Saponins concentration

Sida corymbosa extract (10 g) was dissolved in 20 % ethanol. The mixture was heated at 55 °C in a water bath for 4 h with continuous stirring, and then filtered. The residue obtained was added to 200 mL of 20 % ethanol and filtered. The filtrate was transferred to a 300 mL separatory funnel, then 22 mL of diethyl ether was added and the whole was stirred. The aqueous layer was collected while the ether layer was discarded. The process was once repeated, and 60 mL of n-butanol was added to the recovered aqueous solution. The combined n-butanol and extract was washed twice with 20 mL of 10 % sodium chloride. The remaining solution was evaporated in an oven at 55 °C, and a dried extract was finally obtained. The saponin concentration, expressed as % total saponin, was determined using the following formula [49]:

$$\%\ total\ saponin = [mass\ of\ saponins\ (mg)\ /\ mass\ of\ \boldsymbol{S.\ corymbosa}\ extract\ (\mathbf{g})]^{*}\mathbf{100}.$$

2.9. Statistical analysis

Statistical analysis of obtained data and plotted graphs were performed using Graph Pad Prism version 8.01 software (GraphPad Software, San Diego, CA, USA) and Microsoft Office Excel 2019 (Microsoft Corporation, Redmon, Washington, USA). The results were expressed as the mean ± standard error (SEM) or percentage. The different values were compared using the one-way analysis of variance (ANOVA) followed by the Turkey multiple comparison post hoc test. Differences were considered significant from $p < 0.05$.

3. Results

3.1. Effects of aqueous extract of Sida corymbosa on the latency to first seizures

Fig. 2 illustrates the effects of *S. corymbosa* aqueous extract on the latency to first seizures. The injection of pentylenetetrazole in the negative control group decreased the latency from 1017.5 s ($p < 0.001$) on day 1 to 61.90 s on day 20 compared to the normal control group (Fig. 2). The extract at the doses of 17.14 and 34.28 mg/kg increased this time from 0 s ($p < 0.001$) on day 1 to 660 s ($p < 0.001$)

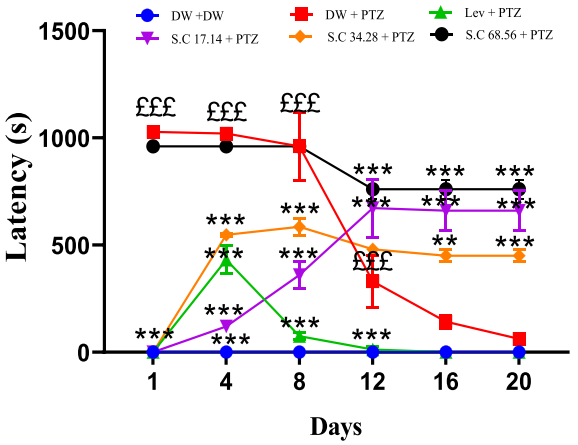


Fig. 2. Effects of aqueous extract of *Sida corymbosa* on the latency to first seizures.
Each value represents the average ± SEM; n = 8. $^{£££}p < 0.001$: significant difference from the normal control group (DW + DW). **p < 0.01; ***p < 0.001: significant difference from the negative control group (DW + PTZ). DW + DW = group receiving distilled water; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

and 450 s ($p < 0.001$) on day 20, respectively. Administration of levetiracetam only increased this time from 0 s ($p < 0.001$) on day 1 to 431.25 s ($p < 0.001$) on day 4 compared to the negative control group (Fig. 2).

These findings indicated that the aqueous extract was more effective than levetiracetam (a new antiepileptic drug) in delaying the onset of the disease.

3.2. Effects of aqueous extract of Sida corymbosa on seizure severity

Fig. 3 shows the effects of *S. corymbosa* on seizure severity. In the negative control group, pentylenetetrazole increased seizure severity from stage 0 on day 1 to stage 5 ($p < 0.001$) on day 20 compared to the normal control group (Fig. 3). The extract, at the dose of 34.28 mg/kg, induced the highest protection by maintaining the seizure severity to stage 0.88 ($p < 0.001$) from day 8 to day 20. Levetiracetam completely prevented seizures ($p < 0.001$) from day 8 to day 20 compared to the negative control group (Fig. 3).

The extract at the dose of 34.28 mg/kg could reduce the severity of seizures in temporal lobe epilepsy patients as levetiracetam (a standard antiepileptic drug). Further studies are needed to confirm these results.

3.3. Effects of aqueous extract of Sida corymbosa on the number of seizures

Fig. 4 shows the effects of the aqueous extract of *S. corymbosa* on the number of seizures. Administration of pentylenetetrazole in the negative control group led to an increase in the number of seizures from 0 on day 1–17.50 ($p < 0.001$) on day 20 compared with the normal control group (Fig. 4). In addition, the extract at a dose of 68.56 mg/kg and levetiracetam prevented seizures ($p < 0.001$) from day 1 to day 12 and limited the number of seizures to 1 ($p < 0.001$) from day 12 to day 20 compared with the negative control group (Fig. 4).

At the highest dose, the extract was more effective than levetiracetam. Although many studies are needed to confirm these results, we can speculate that the extract could be used as an adjuvant to reduce seizure frequency in patients with temporal lobe epilepsy.

3.4. Effects of the aqueous extract of Sida corymbosa on the novel object recognition

Fig. 5 shows the effects of *S. corymbosa* on the novel object recognition. The recognition index in the negative control group decreased by 20 % ($p < 0.001$) compared with the normal control group (Fig. 5). The extract at the doses of 17.14 and 34.28 mg/kg increased by 80 % ($p < 0.05$) and 60 % ($p < 0.01$), respectively. Levetiracetam increased this parameter by 80 % ($p < 0.001$) compared with the negative control group (Fig. 5).

The extract and levetiracetam could improve the recognition memory deficit in patients with temporal lobe epilepsy.

3.5. Effects of the aqueous extract of Sida corymbosa on the time spent in the target quadrant of the morris water maze

Fig. 6 shows the effects of the extract of *S. corymbosa* on the time spent in the target quadrant of the Morris water maze. In the negative control group, pentylenetetrazole decreased by 64 % ($p < 0.001$) the time spent in the target quadrant compared with the

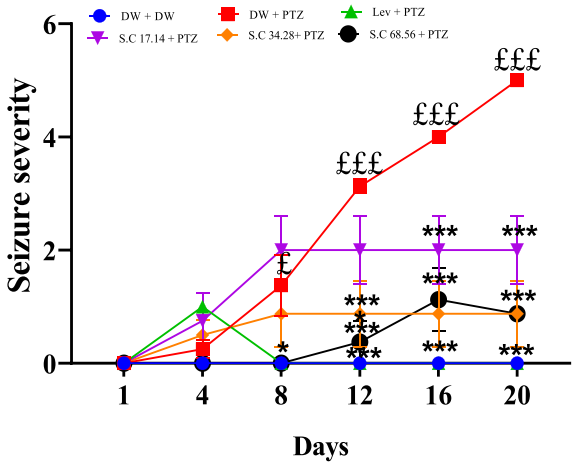


Fig. 3. Effects of aqueous extract of *Sida corymbosa* on seizure severity.
Each point represents the mean ± the SEM of the group, n = 8. £$p < 0.05$, £££$p < 0.001$: significant difference compared to the normal control group (DW + DW). *$p < 0.05$; ***$p < 0.001$: significant difference compared to the negative control group (DW + PTZ). DW + DW = group receiving distilled water; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

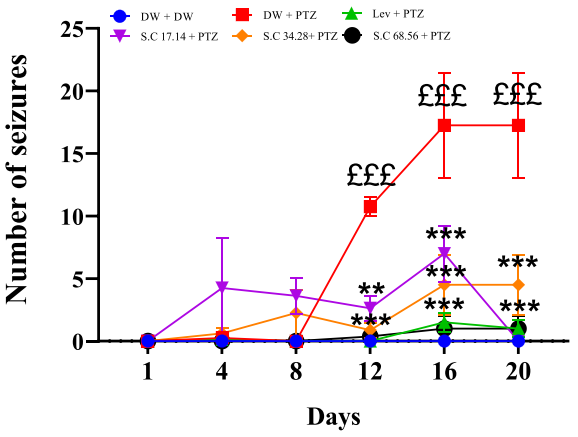


Fig. 4. Effects of aqueous extract of *Sida corymbosa* on the number of seizures.
Each point represents the mean ± the SEM of the group, n = 8. £££p < 0.001: significant difference compared to normal control group (DW + DW); **p < 0.01; ***p < 0.001: significant difference compared to negative control group (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

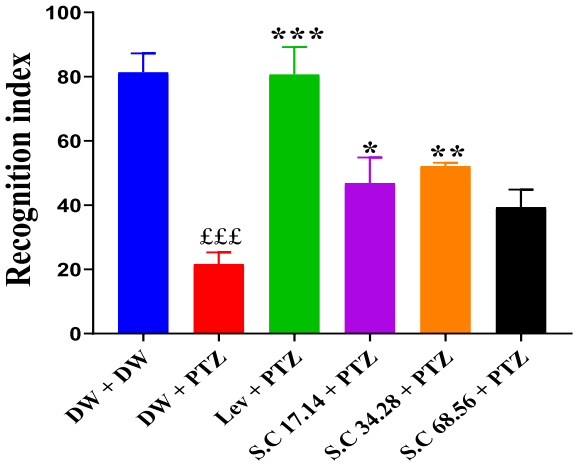


Fig. 5. Effects of the aqueous extract of *Sida corymbosa* on the novel object recognition.
Each bar represents the mean ± the SEM of the group, n = 8. £££p < 0.001: significant difference compared to normal control group (DW + DW). *p < 0.05; **p < 0.01; ***p < 0.001: significant difference compared to negative control group (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

normal control group (Fig. 6). The extract at the doses of 17.14 and 34.28 mg/kg increased this time by 70 % ($p < 0.001$) compared with the negative control group. Levetiracetam increased this time by 57 % ($p < 0.01$) compared with the negative control group (Fig. 6).

The extract at the lowest doses was more effective than levetiracetam in attenuating spatial memory deficit. Thus, the extract could be used as an adjunct to improve spatial memory deficit in patients with temporal lobe epilepsy.

3.6. Effects of the aqueous extract of Sida corymbosa on long-term memory in the Barnes maze

Fig. 7 shows the number of errors (Fig. 7A) and the latency to find the escape box (Fig. 7B). In the negative control group, pentylenetetrazole increased the number of errors ($p < 0.05$) and the latency time to find the escape box (48 %, $p < 0.01$) compared with the normal control group (Fig. 7A and B). The extract at all doses decreased the number of errors ($p < 0.001$) (Fig. 7). However, at a dose of 68.56 mg/kg, it decreased by 70 % ($p < 0.001$) the time to find the escape box. Levetiracetam decreased the number of errors ($p < 0.05$. It also decreased the latency to find the escape box by 61 % ($p < 0.01$) compared with the negative control group (Fig. 7A and B). The extract at the highest dose could attenuate long-term memory impairment in patients with temporal lobe epilepsy when compared to levetiracetam.

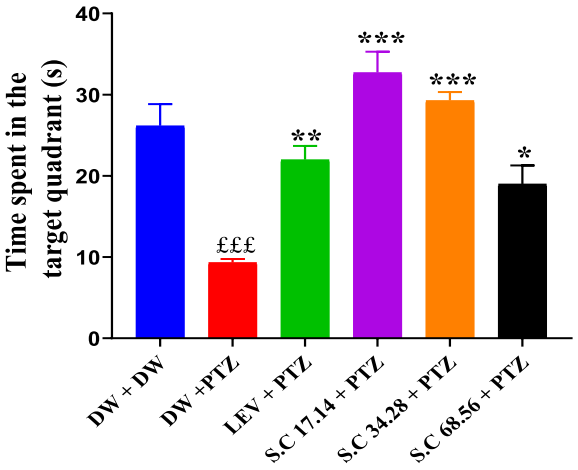


Fig. 6. Effects of the aqueous extract of *Sida corymbosa* on the time spent in the target quadrant of the Morris water maze.
Each bar represents the mean ± the SEM of the group, n = 8. £££p < 0.001: significant difference compared to normal control group (DW + DW). *p < 0.05; **p < 0.01; ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

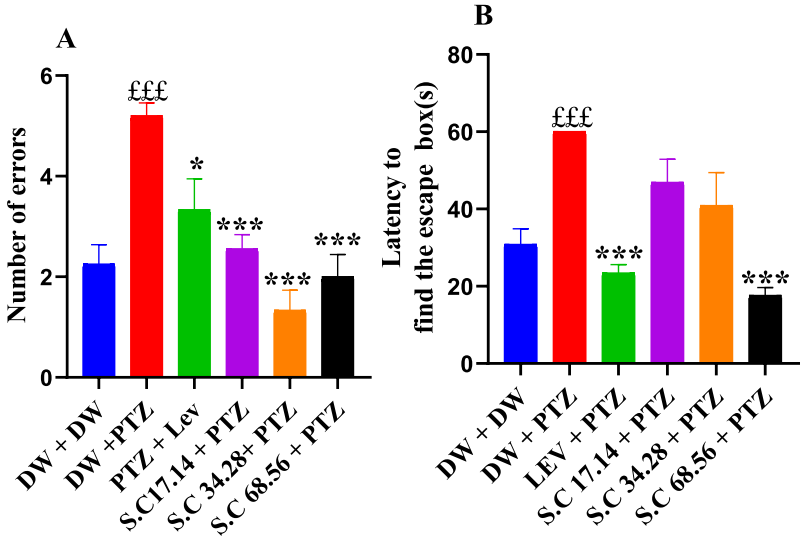


Fig. 7. Effects of the aqueous extract of *Sida corymbosa* on the number of errors (A) and the latency to find the escape box (B) in the Barnes maze.
Each bar represents the mean ± the SEM of the group, n = 8. £££p < 0.001: significant difference compared to normal control group (DW + DW). *p < 0.05; ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

3.7. Effects of the aqueous extract of Sida corymbosa on γ-Aminobutyric acid metabolism

The effects of *S. corymbosa* extract on γ-Aminobutyric acid (GABA) metabolism are shown in Fig. 8. In the negative control group, the concentration of GABA decreased by 61 % ($p < 0.001$) in the hippocampus, while that of L-GAD decreased by 38 % ($p < 0.05$) in the prefrontal cortex and the hippocampus (Fig. 8). The extract at a dose of 17.14 mg/kg increased the concentration of GABA by 68 % ($p < 0.001$)) in the hippocampus, while at a dose of 68.56 mg/kg, it increased the L-GAD activity by 68 % ($p < 0.01$) in the same brain structure (Fig. 8). Levetiracetam increased these parameters by 45 % ($p < 0.01$) in the hippocampus and by 81 % ($p < 0.001$) in the prefrontal cortex compared with the negative control group (Fig. 8).

Compared to levetiracetam, the extract at the highest dose could be used to increase the GABA level in the brain of patients with

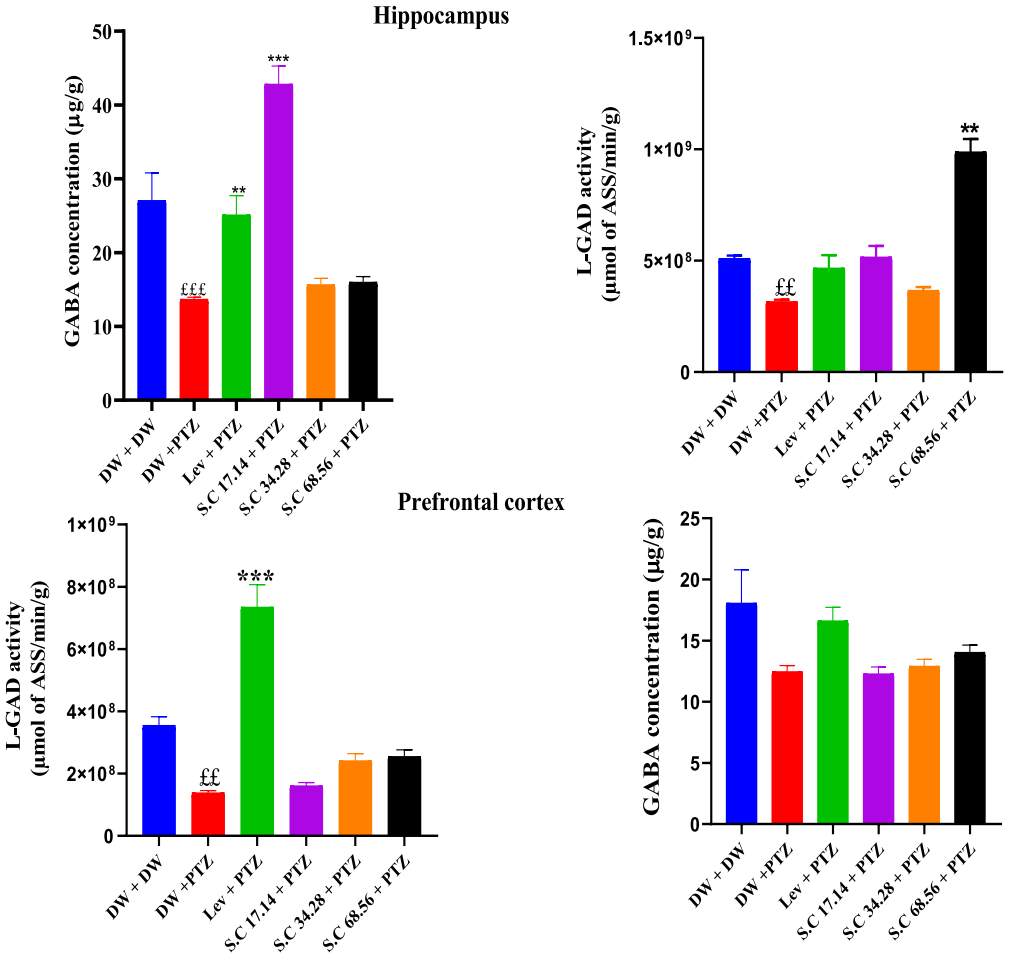


Fig. 8. Effects of the aqueous extract of *Sida corymbosa* on the γ-Aminobutyric acid concentration and L-glutamate decarboxylase activity in the hippocampus and prefrontal cortex.
Each bar represents the mean ± the SEM of the group, n = 8. ££p < 0.01; £££p < 0.001: significant difference compared to normal control group (DW + DW). **p < 0.01; ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

temporal lobe epilepsy.

3.8. Effects of the aqueous extract of Sida corymbosa on acetylcholine concentration

Fig. 9 summarizes the effects of the aqueous extract of *S. corymbosa* on acetylcholine concentration. Administration of pentylenetetrazole in the negative control group resulted in a decrease in acetylcholine concentration by 85 % ($p < 0.001$) in the prefrontal cortex and by 74 % ($p < 0.05$) in the hippocampus compared with normal control group (Fig. 9). The extract at all doses increased the acetylcholine level ($p < 0.001$) in the prefrontal cortex. However, the extract (17.14 and 34.28 mg/kg) increased this parameter by 51 % ($p < 0.001$) and 69 % ($p < 0.001$) in the hippocampus, respectively (Fig. 9). Levetiracetam increased the concentration of acetylcholine by 43 % ($p < 0.01$) and 71 % ($p < 0.001$) in the hippocampus and prefrontal cortex, respectively, compared with the negative control group (Fig. 9).

The extract, at the lowest doses, could improve brain acetylcholine levels in the brain of patients with temporal lobe epilepsy.

3.9. Effects of the aqueous extract of Sida corymbosa on some oxidative stress markers

Fig. 10 shows the effects of *S. corymbosa* on some parameters of oxidative stress in the prefrontal cortex and hippocampus. In the negative control group, pentylenetetrazole increased by 39 % ($p < 0.001$) the concentration of MDA concentration in the prefrontal

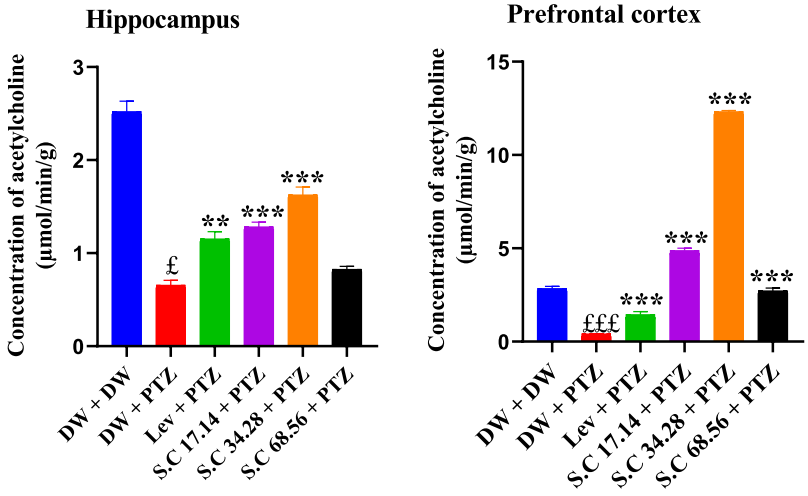


Fig. 9. Effects of the aqueous extract of *Sida corymbosa* on the concentration of acetylcholine in the hippocampus and prefrontal cortex.
Each bar represents the mean ± the SEM of the group, n = 8. £p < 0.05; £££p < 0.001: significant difference compared to normal control group (DW + DW). **p < 0.01, ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole; Ach: Acetylcholine.

cortex and hippocampus compared with the normal control group. The extract at a dose of 17.14 mg/kg decreased the concentration of MDA in the hippocampus by 43 % (p < 0.001) (Fig. 10). The extract at the doses of 17.14 mg/kg and 34.28 mg/kg decreased the concentration of MDA by 5 % (p < 0.01) in the prefrontal cortex compared with the negative control group. Levetiracetam decreased this concentration by 28 % (p < 0.001) and 67 % in the hippocampus and prefrontal cortex, respectively, compared to the negative control group (Fig. 10).

Pentylenetetrazole decreased the GSH concentration in the hippocampus and prefrontal cortex of the negative control group by 69 % (p < 0.01) and 80 % (p < 0.001), respectively, compared to the normal control group (Fig. 10). The extract at the doses of 17.14 mg/kg and 34.28 mg/kg increased this parameter by 51 % (p < 0.05) and 55 % (p < 0.01) in the hippocampus, respectively. In the prefrontal cortex, the extract (17.14 and 68.56 mg/kg) also increased the concentration of GSH by 88 % (p < 0.001) and 85 % (p < 0.001), respectively (Fig. 10). Levetiracetam increased by 75 % (p < 0.05) the concentration of GSH in the prefrontal cortex compared with the negative control group (Fig. 10).

The extract was endowed with strong antioxidant activity. Therefore, it could be used as an adjunct to improve the quality of life of patients with temporal lobe epilepsy.

3.10. Effects of the aqueous extract of Sida corymbosa on the hippocampal microarchitecture

Histological analysis of the hippocampus revealed, in the normal control group, a normal structure of the hippocampal parenchyma (Figs. 11 and 12), with apparently intact neurons in the different layers of the hippocampus (CA1, CA2, CA3, and dentate gyrus). In the negative control group, pentylenetetrazole caused the loss of neurons, marked by the presence of neurons with pycnosate (hyperchromatic) nuclei, and cytolysis (Figs. 11 and 12). The extract at all doses and levetiracetam showed a microstructure close to that of the normal control group (Figs. 11 and 12).

The extract could help to prevent or attenuate brain injury in patients with temporal lobe epilepsy associated with hippocampal sclerosis.

3.11. Effects of aqueous extract of Sida corymbosa on hippocampal neuron density

In the negative control group, pentylenetetrazole decreased the number of living neurons by 58 % (p < 0.001) in the CA3 and dentate gyrus of the hippocampus compared with the normal control group (Fig. 13). The extract at the highest dose increased the number of living neurons in the CA1 et CA2 regions by 50 % (p < 0.001), while in the CA3 and dentate gyrus regions, the extract at all doses increased (p < 0.001) this number (Fig. 13). Levetiracetam caused a 44 % (p < 0.001) increase in this number in the CA3 region compared with the negative control group (Fig. 13).

The extract at all doses could attenuate neuronal loss in the hippocampus of patients with temporal lobe epilepsy.

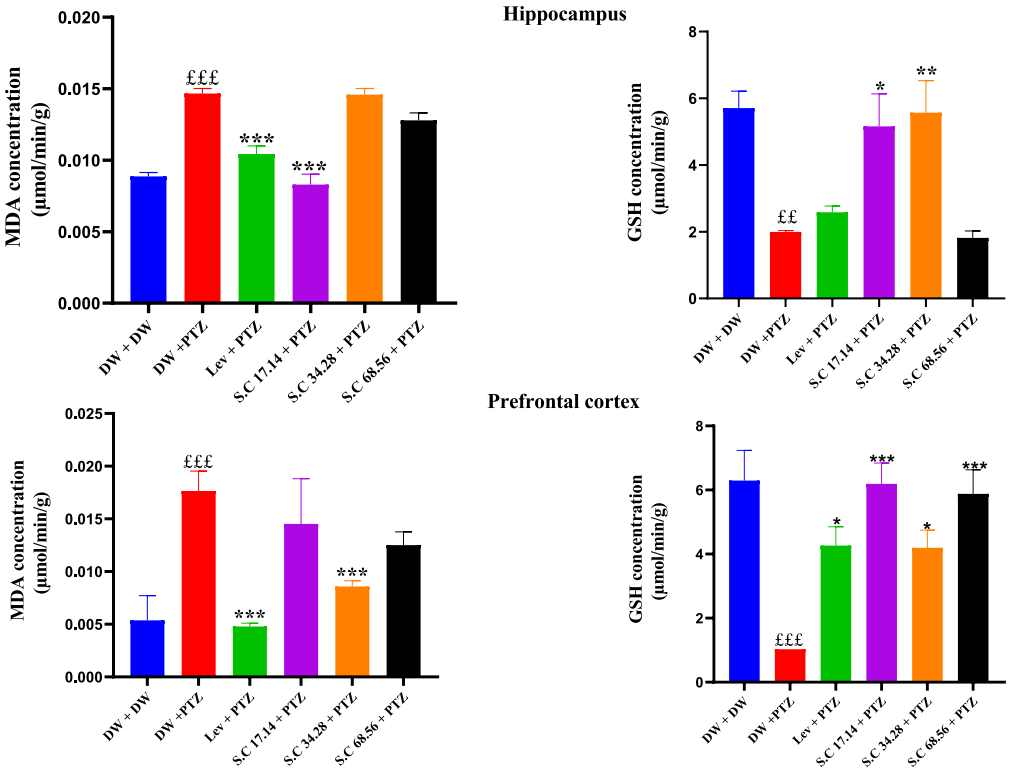


Fig. 10. Effects of the aqueous extract of *Sida corymbosa* on malondialdehyde and reduced glutathione concentrations in the prefrontal cortex and hippocampus.
Each bar represents the mean ± the SEM of the group, n = 8. ££p < 0.01; £££p < 0.001: significant difference compared to normal control group (DW + DW). *p < 0.05; **p < 0.01; ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

3.12. Quantitative phytochemical analysis of the aqueous extract of Sida corymbosa

It is of interest to quantify bioactive compounds as they are known to interact with pathological processes. Indeed, alkaloids can modulate the GABAergic system, phenolic compounds have strong antioxidant and anti-inflammatory properties, saponins can modulate calcium and sodium functions, tannins can regulate apoptosis and inflammation, and finally flavonoids can interact with synaptic functions to inhibit abnormal neural depolarization in epilepsy. In the present findings, the aqueous extract of *S. corymbosa* was found to have 269.31 ± 0.24 mg gallic acid equivalent/g of phenolic compounds, 50.13 ± 0.19 mg catechin equivalent/g of condensed tannins, 39.73 ± 0.35 mg rutin equivalent/g of total flavonoids, 10.11 ± 0.26 % of total alkaloids, and 5.95 ± 0.41 % of saponins.

4. Discussion

Epilepsy is a chronic brain disorder characterized by abnormal and sustained electrical activity in the brain [2]. An epileptic seizure is the clinical manifestation of paroxysmal, hypersynchronous, and self-sustained discharge of a more or less extensive population of neurons [39]. The present study aimed to evaluate the antiepileptic and anti-amnestic effects of the aqueous extract of *Sida corymbosa* using the PTZ kindling model in rats. Repeated administration of PTZ (25 mg/kg) resulted in a progressive onset of seizures from stage 1 to stage 5, a decrease in seizure onset time, and an increase in the number of seizures. PTZ induces epileptic seizures in rodents by blocking the chlorine channel of GABAA receptors [7]. During an action potential, the chlorine ion (Cl^-) is no longer in equilibrium, and there is an influx of Cl^- ion into the cell, which tends to oppose depolarization [37,50]. At a subconvulsive dose, chronic administration of PTZ leads to the development of seizures, initially manifesting as weak myoclonic jerks, then evolving into generalized tonic-clonic seizures [37,50]. Administration of the aqueous extract of *S. corymbosa* significantly increased the onset time and duration of seizures and decreased the number of epileptic seizures compared with the negative control group. These findings

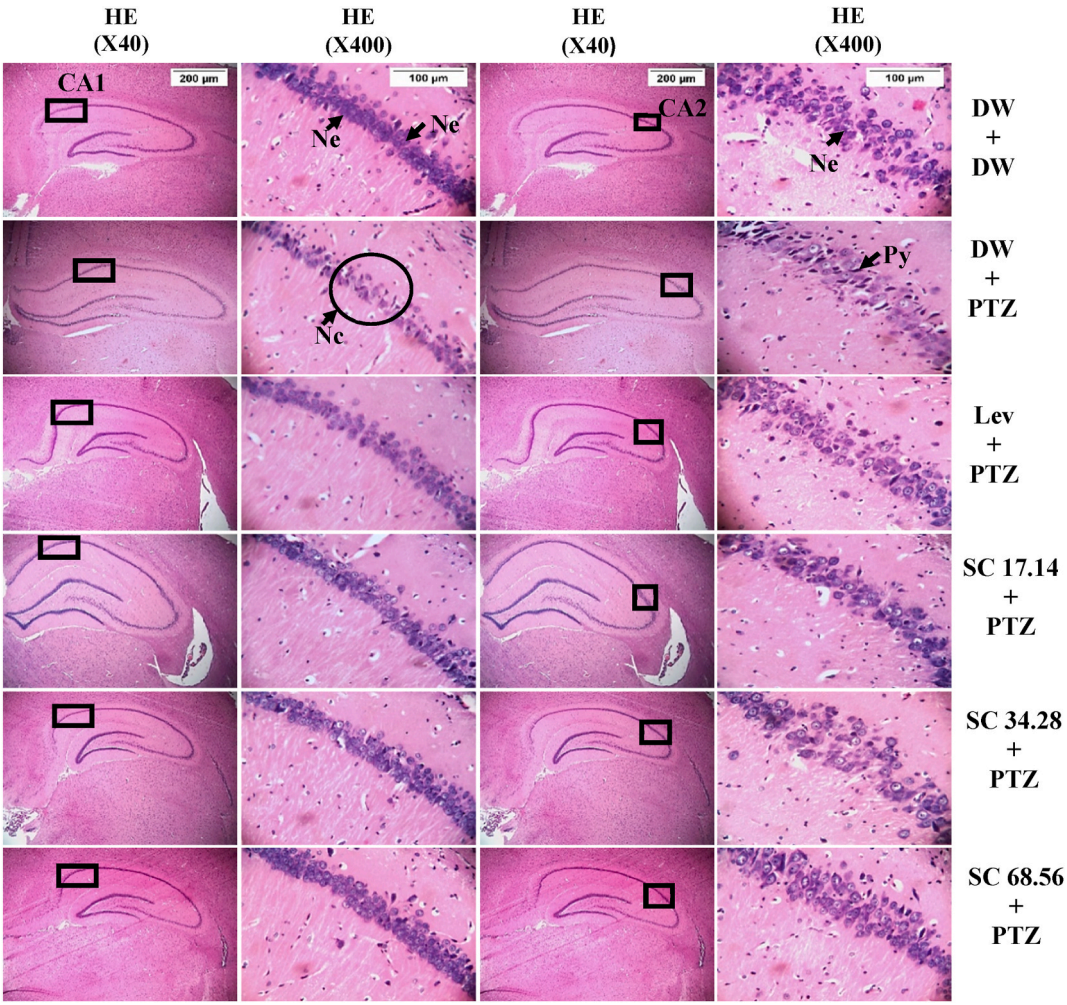


Fig. 11. Microphotographs of CA1 and CA2 of the hippocampus (Hematoxylin-eosin staining).
DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole; Ne = normal neuron; Cn = condensed nucleus; Nc = Neuronal cytolysis or loss; Py = Pyknosis.

suggest that the extract possesses an antiepileptic effect when compared with levetiracetam, a reference antiepileptic drug. These effects could be due to an action of the extract on the symptoms or underlying mechanisms of seizures. The presence of saponins and flavonoids in the extract could explain in part these properties [51,52]. Saponins and flavonoids have been shown to inhibit epileptic seizures by antagonizing glutamatergic receptors or potentiating the $GABA_A$ receptor complex *in vivo* and *in vitro* models of seizures [53].

Temporal lobe epilepsy is often associated with impaired memory function [54]. In the present study, pentylenetetrazole kindling resulted in a significant decrease in the recognition index, a reduction in the time spent in the target quadrant in the Morris water maze, and an increase in latency to finding the escape box coupled with an increase in the number of errors in the Barnes maze. This variation in behavioral parameters assessed in the Morris water maze, Barnes circular maze, and novel object recognition tests suggests impaired short-term and long-term memories [54]. This could be due to structural or functional alterations in the hippocampus and prefrontal cortex [37,55]. Administration of the aqueous extract of *S. corymbosa* compared with the negative control reversed the variation in the above-mentioned behavioral parameters, suggesting that the extract is endowed with an anti-amnestic activity. This activity could be the consequence of flavonoid activity. Indeed, according to Uriarte and Calvo [55], flavonoids have been shown to inhibit acetyl-choline esterase activity.

In the brain, GABA synthesis is under the control of the enzyme L-GAD [56]. In the present work, a decrease in GABA and L-GAD levels was observed in the hippocampus or prefrontal cortex of the negative control group compared with the normal control group. However, an increase in GABA levels was noted in the hippocampus or prefrontal cortex of animals treated with *S. corymbosa* extract. This increase seems to be attributed in part to increased L-GAD activity [56]. The metabolites found in *S. corymbosa* aqueous extract are known to potentiate GABAergic neurotransmission. Saponins and flavonoids for example have been shown to enhance GABAergic

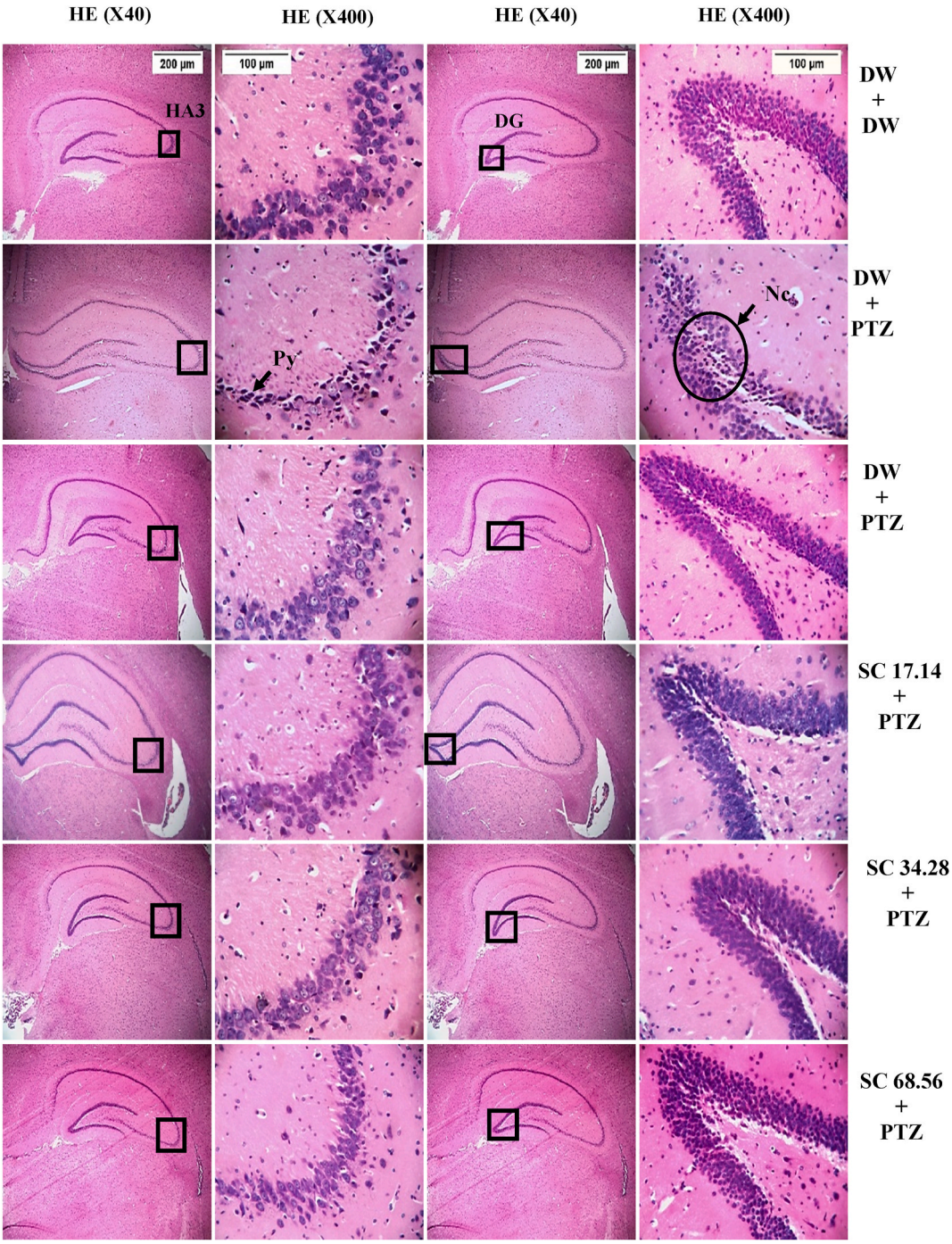


Fig. 12. Microphotographs of the CA3 and the dentate gyrus of the hippocampus (hematoxylin-eosin staining).
DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole; Ne = neuron; Pn = Neuronal cytolysis and loss; Py = Pyknosis.

neurotransmission, while polyphenols have been shown to stimulate L-GAD activity [57,58].

Analysis of oxidative stress parameters showed that repeated injections of pentylenetetrazole resulted in oxidative stress in the form of increased levels of malondialdehyde (MDA), as well as reduced glutathione (GSH) in the hippocampus and prefrontal cortex of the negative control group. Pentylenetetrazole indirectly stimulates the glutamatergic system, which in turn generates tonic-clonic seizures [7]. This excitotoxicity is at the root of ROS production, leading to MDA-generating lipid peroxidation and a drop in GSH levels

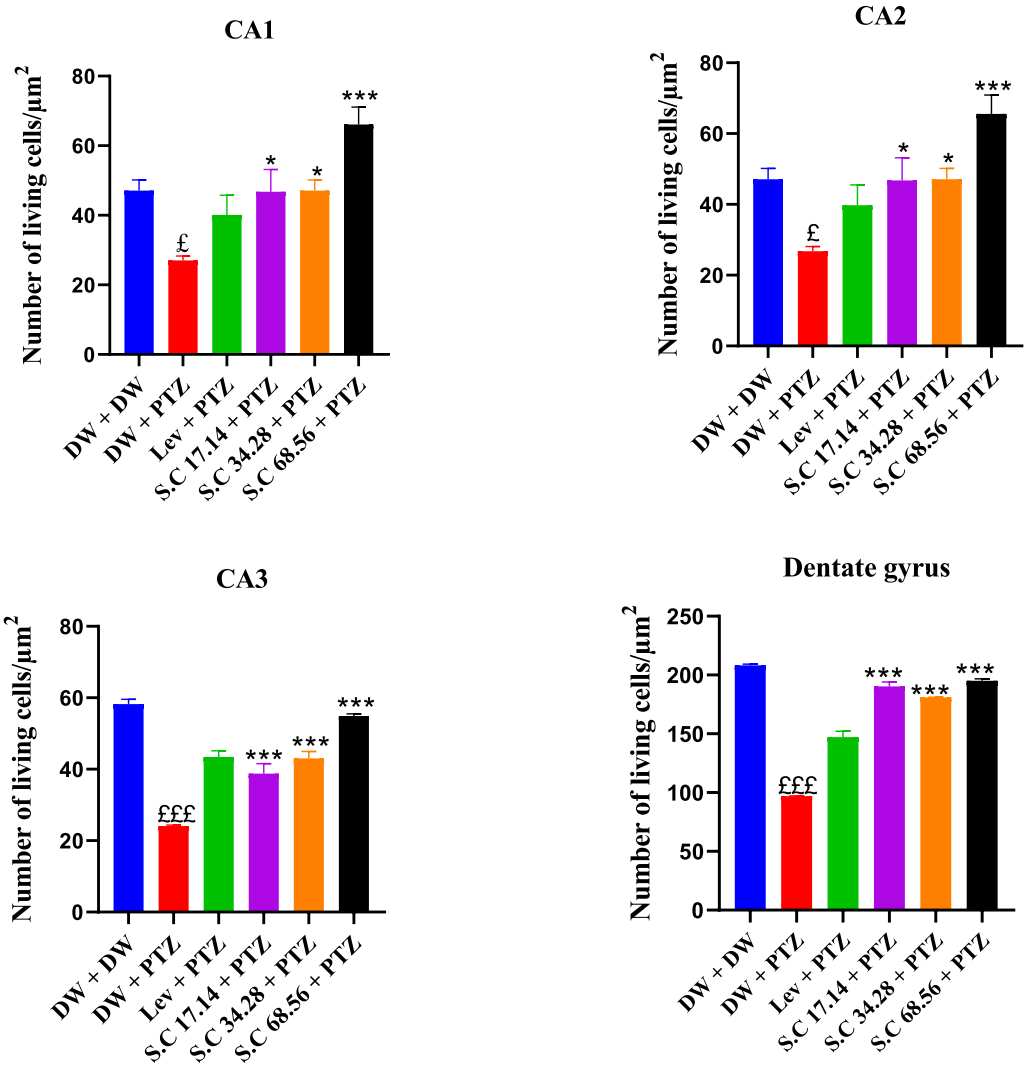


Fig. 13. Effects of the aqueous extract of *Sida corymbosa* on neuron density. Each bar represents the mean ± the SEM of the group, n = 8. £p < 0.05; £££p < 0.001: significant difference compared to normal control group (DW + DW). *p < 0.01; ***p < 0.001: significant difference compared to negative control (DW + PTZ). DW + DW = group receiving distilled water only; DW + PTZ = group receiving distilled water and pentylenetetrazole; Lev + PTZ = group receiving levetiracetam and pentylenetetrazole; S.C (17.14, 34.28, and 68.56) + PTZ = groups treated with extract at respective doses of 17.14, 34.28, and 68.56 mg/kg and pentylenetetrazole.

due to its use during the neutralization of free radicals by glutathione peroxidase [7]. These results corroborate those of Kandeda et al. [7] who showed that the injection of pentylenetetrazole into rodents is associated with strong oxidative stress in the brains of rodents. Indeed, glutamate binding to its receptors leads to an influx of calcium ions (Ca^{2+}) into the cell. This influx of Ca^{2+} ions activates numerous enzymes (lipases, proteases, and endonucleases) and increases the production of reactive oxygen species (ROS) [59]. These actions damage the cell, leading to neuronal death. Oxidative stress-induced damage is thought to play an important role in the pathogenesis of various neurological and behavioral disorders [59]. The aqueous extract of *S. corymbosa* improved cerebral oxidative status by decreasing MDA level and increasing that of GSH in brain tissue, suggesting that the plant extract is endowed with antioxidant activity. This antioxidant property of *S. corymbosa* could be explained by the presence of secondary metabolites such as flavonoids and phenolic compounds [56,60], which are reputed to possess antioxidant power. Moreover, bioactive compounds are said to increase eNOS activity and reduce NADPH-oxidase expression [61]. These two processes are thought to contribute to the inhibition of free radicals in the brain [61].

Behavioral alterations such as amnesia are thought to be the result of histological alterations in the brain [52]. Pentylenetetrazole kindling is known to induce neuronal death, leading to cognitive function impairment [7]. In the present study, PTZ induced neurofibrillary degeneration, marked by a loss of neuronal cells in CA1, CA2, CA3, and dentate gyrus regions of the hippocampus.

These results are in line with the works of Kandeda et al. [7] and Taiwe et al. [61], who showed hippocampal disorganization during epilepsy in rodents. The aqueous extract and levetiracetam protected animals from the harmful effects of PTZ. This was characterized by an improvement in the hippocampal neuron density compared with the negative control group. These results suggest the neuroprotective properties of the extract. Numerous studies revealed that flavonoids can slow down the progression of neurodegenerative diseases by inhibiting neuronal apoptosis or necrosis [60]. Further studies have to be performed to unravel the mechanisms involved in the neuroprotective effects of the extract.

Chemical compounds deriving from plants revealed to possess anticonvulsant activity in *in vitro* and *in vivo* assays [62]. Anticonvulsant properties of the extract from plants may involve terpenoids, flavonoids, phenylpropanoids, phenolic compounds, saponins, coumarins, and flavonoids [62]. These bioactive compounds have been shown to interact with the voltage-gated ion channels (calcium and sodium), the $GABA_A$ receptor complex, and the metabolism of GABA (inhibition of GABA-transaminase and stimulation of L-GAD) [62]. Modulation of these pathways explains the antiepileptic and anticonvulsant potential of secondary metabolites from plants [62]. Furthermore, bioactive compounds from medicinal plants showed neuroprotective, antioxidant, memory-enhancing, and anti-inflammatory activities that contribute in part to the antiepileptic effects of plant-derived extract [62]. Therefore, Cameroonian flora provide a basis for target-oriented antiepileptic drug discovery.

Quantitative phytochemical analysis of the aqueous extract indicated a significant presence of phenolic compounds, condensed tannins, and total flavonoids compared to the findings obtained by Djeuzong et al. [43], Dehpour et al. [45], and Mang' Dobara et al. [46] suggested that the antiepileptic and anti-amnesic effects of the extract could be related to the abundance of these compounds [57]. According to the scientific literature, flavonoids can modulate the GABAergic neurotransmission system and interact with synaptic functions to inhibit abnormal neuronal depolarization in epilepsy [57], phenolic compounds can attenuate or prevent oxidative stress and inflammation [58], and tannins can regulate apoptosis, necrosis, and inflammation in experimental models of epilepsy [63]. Besides, the low concentration of alkaloids and saponins in the extract, when compared to the results of studies performed by Gracelin et al. [48] and Djeuzong et al. [43], does not mean that these compounds have little or no impact, as saponins have been shown to modulate calcium and sodium functions [64], even at low concentrations [64], while alkaloids are thought to act in synergy with other bioactive compounds to modulate $GABA_A$ receptor complex functions [65]. In the present state of knowledge, it is not possible to attribute with certainty the antiepileptic and memory-enhancing properties of the aqueous extract to one or several bioactive compounds [66,67]. In addition, we cannot confirm that the antiepileptic and memory-enhancing activities of the extract are linked to the abundance of phenolic compounds, condensed tannins, and flavonoids. The main limitation of this study is therefore the lack of the metabolite composition of the aqueous extract of *S. corymbosa*. This is related to knowing what metabolites or mix of them could cross the blood-brain barrier to exert the beneficial effect mentioned in the present study, and equally to know which metabolites could exert adverse effects. Further studies should be performed to address these limits and unravel the exact mechanisms by which the extract induced antiepileptic and anti-amnesic effects. Considering the potentially useful therapeutic effects of the extract, studies are needed to justify its human consumption.

5. Conclusion

The present study aimed to evaluate the antiepileptic and anti-amnesic effects of the aqueous extract of *Sida corymbosa* using the PTZ-kindling model in rats. Treatment with an extract of *Sida corymbosa* attenuated the severity and number of seizures. This treatment also attenuated memory impairment following PTZ-kindling. Biochemical analyses revealed that the extract increased the concentrations of GABA and acetylcholine, and decreased that of malondialdehyde in the hippocampus and prefrontal cortex. Finally, the extract showed neuroprotective activity by attenuating neuronal damage induced by PTZ-kindling in the hippocampus. All these findings suggest that the extract possesses antiepileptic, anti-amnesic, antioxidant, and neuroprotective effects. This justifies its use in traditional medicine for the treatment of epilepsy and other neurological diseases.

CRediT authorship contribution statement

Marguerite Zizanie Penda: Writing – original draft, Software, Methodology, Investigation, Data curation. **Antoine Kavaye Kandeda:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Frida Longo:** Writing – original draft, Validation, Supervision, Methodology, Data curation, Conceptualization. **Livana Brinda Ateufack:** Methodology, Investigation, Formal analysis. **Théophile Dimo:** Validation, Supervision.

Ethics statement

Animal procedures were carried out following the guidelines of the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technological Innovation (Reg. no. FWA-IRD 0001954, September 04, 2006), which adopted the guidelines of the European Union on Animal Care (C.E.E. Council 86/609).

Funding statement

This study did not receive any financial or commercial support.

Data availability

No data associated with our study has been deposited into a publicly available repository. However, the data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the Laboratory of Animal Physiology (University of Yaoundé I, Cameroon).

References

[1] WHO, Framework for Strengthening the Implementation of the Global Action Plan for Mental Health2013 – 2030in the WHO African Region: Secretariat Report, Organisatiuon Mondiale de la Santé. Bureau Régional de l'Afrique, 2022.
[2] X. Mao, Jin W. Zhou, Ferroptosis induction in pentylenetetrazole kindling and pilocarpine-induced epileptic seizure in mice, Neuroscience (2019) 13–721.
[3] L.A. Rudzinski, K.J. Meador, Epilepsy and neuropsychological comorbidities, Continuum: Lancet Neurol. 19 (3) (2013) 682–696.
[4] A. Lazarowski, L. Czornyj, Potential role of multidrug-resistant proteins in refractory epilepsy and antiepileptic drugs interactions, Drug Metabolism, Drug Interaction 26 (1) (2011) 21–26.
[5] Bappa A. Lukman, M. Abubakar, D. Shakira, Prévalence of active epilepsy prevalence, and burden of epilepsy in sub-Saharan Africa from a meta-analysis of door-to-door population-based surveys, Epilepsy Behav. 103 (2020) 106–846.
[6] A.K. Kandeda, S. Menvouta, S.T. Mabou, J. Kouamouo, T. Dimo, Aqueous extract of Parkia biglobosa (Jacq.) R. Br.(Fabaceae) exerts antiepileptogenic, anti-amnesic, and anxiolytic-like effects in mice via mechanisms involving antioxidant and anti-inflammatory pathways, Fronti. Pharmacol. 13 (2022) 995881.
[7] K.A. Kandeda, O.F.C. Moto, M.R.E. Ayissi, O.J.P. Omam, Ojong c Lucie, Bum E. Ngo, *Pergularia daemia* hydro-ethanolic extract protects against pentylenetetrazole kindling-induced seizures, oxidative stress, and neuroinflammation in mice, J. Ethnopharmacol. 279 (2021) 1–12.
[8] W. Loscher, D. Schmidt, New horizons in the development of antiepileptic drugs, Epilepsy Res. 50 (1) (2002) 3–16.
[9] P. Kandasamy, G. Gyimesi, Y. Kanai, M.A. Hediger, Amino acid transporters revisited: new views in health and disease. Trends Biochemical, Sciences 43 (10) (2018) 752–789.
[10] A.K. Kandeda, G.S. Taiwe, F.C. Moto, G.T. Ngoupaye, G.C. Nkantchoua, J.S. Njapdounke, O.J.P. Omam, S. Pale, N. Kouemou, E. Ngo Bum, Antiepileptogenic and neuroprotective effects of Pergularia daemia on pilocarpine model of epilepsy, Front. Pharmacol. 8 (2017) 440.
[11] R.A. Floyd, Neuroinflammatory processes are important in neurodegenerative diseases: a hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development, Free Radic. Biol. Med. 26 (9) (1999) 1346–1355.
[12] J.A. Knight, Reactive oxygen species and neurodegenerative disorders, ACLS (Ann. Clin. Lab. Sci.) 27 (1) (1997) 11–25.
[13] J. Mehla, K. Reeta, P. Gupta, Y.K. Gupta, Protective effect of curcumin against seizures and cognitive impairment in a pentylenetetrazole-kindled epileptic rat model, Life Sci. 87 (19) (2010) 596–603.
[14] S.L. Hansen, B.B. Sperling, C. Sanchez, Anticonvulsant and antiepileptogenic effects of GABAA receptor ligands in pentylenetrétrazole-kindling mice, Progress in Neuro-psychopharmacology and Biological Psychiatry 28 (1) (2004) 105–113.
[15] K. Morimoto, M. Fahnestock, R.J. Racine, Kindling and status epilepticus models of epilepsy: rewiring the brain, Prog. Neurobiol. 73 (1) (2004) 1–60.
[16] J.O. McNamara, M.C. Byrne, R.M. Dasheiff, J.G. Fitz, The kindling model of epilepsy: a review, Prog. Neurobiol. 15 (2) (1980) 139–159.
[17] N. Bernasconi, A. Bernasconi, Z. Caramanos, S. Antel, F. Andermann, D. Arnold, Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala, and parahippocampal region, Brain 126 (2) (2003) 462–469.
[18] L. Bernardino, R. Ferreira, Sales F. Cristovao, J.O. Malva, Inflammation and neurogenesis in temporal lobe epilepsy, Curr. Drug Targets - CNS Neurol. Disord. 4 (4) (2005) 349–360.
[19] A.B. Muhammad, H.S. Adegoke, I.M. Samaila, S.C. Musa, Stem bark chloroform extract of *Bombax costatum* Pellegr. & Vuillet exhibit anticonvulsant and neuroprotective effects in pentylenetetrazole-induced seizures in rats, Ann. Pharm. Fr. (2023) 233–247.
[20] G.L. Holmes, Seizure-induced neuronal injury animal data, Neurology 59 (9) (2002) 3–6.
[21] A. Pitkanen, T.P. Sutula, Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy, Lancet Neurol. 1 (3) (2002) 173–181.
[22] K. Majak, A. Pitkanen, Do seizures cause irreversible cognitive damage? Evidence from animal studies, Epilepsy Behav. 5 (2004) 35–44.
[23] J.M. Bakut, Y.A. Tende, A.I. Agyigra, M.O. Tende, A.U. Zezi, N.M. Danjuma, Preliminary phytochemical and toxicity studies of aqueous leaf extract of *Sida corymbosa* plant, Am. J. Plant Sci. 11 (12) (2020) 1991.
[24] L.B. John-Africa, T.A. Yahaya, C.Y. Isimi, Anti-ulcer and wound healing activities of *Sida corymbosa* in rats, Afr. J. Tradit., Complementary Altern. Med. 11 (2013) 87–92.
[25] C.C. Dike, F.C. Ezeonu, H.C.C. Maduk, E.N. Ezeokafor, A.N. Okpogba, E.C. Oguazu, O.A. Eyeghre, S.J. Asebioyo, The effects of *Sida corymbosa* leaf extract against abnormal bilirubin and total protein in albino rats by carbon tetrachloride (CCl4), J. Dis. Med. Plants 6 (2020) 39–46.
[26] C.C. Dike, F.C. Ezeonu, H.C.C. Maduka, E.N. Ezeokafor, Phytochemical and elemental analysis of *Sida corymbosa* (Broom weed or Wire weed) leaf and root extract, Chem. Sci. J. 24 (2018) 1–9.
[27] N.S.Y. Mengue, P. Owona, M. Noubom, M.A. Mbock, N.L. Mbolang, F.R. Ngapout, A.G.R. Bidingha, T.R.B. Kahou, C.D. Bilanda, P. Kamtchoiung, D.P.D. Dzeufiet, Estrogenic and antioxidant activities of *Pterocarpus soyauxii* aqueous extract in bilateral oophorectomized Wistar rat, Evidence-Based Complementary and Alternative Medecine (2021) 1–18.
[28] A. Dhir, Pentylenetetrazol (PTZ) kindling model of epilepsy, Current Protocol in Neuroscience 58 (1) (2012) 31–39.
[29] R.J. Racine, Modification of seizure activity by electrical stimulation: II. Motor seizure, Clin. Neurophysiol. 32 (3) (1972) 281–294.
[30] K.A. Kandeda, G.S. Taiwe, F.C. Moto, G.T. Ngoupaye, G.C. Nkantchoua, J.S. Njapdounke, J.P. Omam, S. Pale, N. Kouemou, Bum E. Ngo, Antiepileptogenic and neuroprotective effects of *Pergularia daemia* on pilocarpine model of epilepsy, Front. Pharmacol. 8 (2017) 440.
[31] R. Aguiar, E. Dickel, W. Cunha, M. Monserrat, M. Barros, E. Martinez, Estradiol valerate and tibolone: effects on memory, Pharmacol., Biochem. Behav. 85 (2006) 689–696.
[32] C.V. Vorhees, M.T. Williams, Morris water maze: procedures for assessing spatial and related forms of learning and memory, Nat. Protoc. 1 (2006) 84–858.
[33] B.C. Mouzon, C. Bachmeier, A. Ferro, O.J. Olubunmi, C. Gogce, Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model, Ann. Neurol. 75 (2) (2014) 241–254.
[34] C.A. Barnes, Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat, J. Comp. Physiol. Psychol. 93 (1) (1979) 74.
[35] F.E. Harrison, A.H. Hosseini, M.P. McDonald, Endogenous anxiety and stress responses in water maze spatial memory tasks, Behav. Brain Res. 198 (1) (2009) 247–251.

[36] S.R. Das, D. Mechanic-Hamilton, M. Korczykowski, J. Pluta, S. Glynn, B.B. Avants, J.A. Detre, P.A.J.H. Yushkevich, Structure-specific analysis of the hippocampus in temporal lobe epilepsy, Hippocampus 19 (2009) 517–525.
[37] K.A. Kandeda, L.B. Stephanie, E.B. Djeuzong, Dimo T. Kouamouo, An aqueous extract of *Khaya senegalensis* (Desv.) A. Juss. (Meliaceae) prevents seizures and reduces anxiety in kainate-treated rats: modulation of GABA neurotransmission, oxidative stress, and neuronal loss in the hippocampus, Heliyon 8 (2022) e09549, 1-17.
[38] Irene P. Lowe, Robins Eli, S. Geraldine, Eyerman, The fluorimetric measurement of glutamic decarboxylase and its distribution in the brain, J. Neurochem. 3 (1) (1958) 8–18.
[39] P. Nayak, A.K. Chatterjee, Effects of aluminum exposure on brain glutamate and GABA systems: an experimental study in rats, Food Chem. Toxicol. 39 (12) (2001) 1285–1289.
[40] S. Hestrin, The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application, Journal of Biology and Chemistry 180 (1) (1949) 249–261.
[41] K. Wilbur, F. Bernheim, O. Shapiro, Determination of lipid peroxidation, Arch. Biochem. Biophys. 24 (1949) 305–310.
[42] I. Rahman, A. Kode, S.K. Biswas, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, Nat. Protoc. 1 (6) (2006) 3159–3165.
[43] E. Djeuzong, K. Kandeda, S. Djiogue, L. Stéphanie, D. Nguedia, F. Ngueguim, T. Dimo, Antiamnesic and neuroprotective effects of an aqueous extract of *Ziziphus jujuba* mill.(Rhamnaceae) on scopolamine-induced cognitive impairments in rats, Evid. base Compl. Alternative Med. 2021 (1) (2021) 5577163.
[44] S.A. Hatami, S.S. Emami, Mojarrab MJ. Miraghaee, Total phenolic contents and antioxidant activities of different extracts and fractions from the aerial parts of *Artemisia biennis* Willd, Iran. J. Pharm. Res. (IJPR) 13 (2) (2024) 551–2014.
[45] A.A. Dehpour, M.A. Ebrahimzadeh, N.S. Fazel, N.S. Mohammad, Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition, Grasas Aceites 60 (4) (2009) 405–412, 2009.
[46] K. Mang'Dobara, N. Francis, M. Raymond, M.N. Bertrand, N.E. Nchiwan, N. Dieudonne, Anti-onchocercal activity and i*n vivo* toxicity of methanolic extract of *Canarium schweinfurthii* (Burseraceae) from the equatorial region (Cameroon), Cameroon Journal of Biological Sciences 28 (2) (2020) 14–25.
[47] K. Ba, E. Tine, J. Destain, N. Cissé, P.J.B. Thonart, Agronomie, Société et Environnement. Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt, Base 14 (1) (2010) 131–139.
[48] Britto A. Gracelin, B.J. Kumar, Qualitative and quantitative analysis of phytochemicals in five Pteris species, Int. J. Pharm. Pharmaceut. Sci. 5 (1) (2013) 105–107.
[49] K.A. Kandeda, D. Nguedia, E. Djeuzong, J. Kouamouo, T. Dimo, An aqueous macerate of *Ziziphus jujuba* reduces long-term spatial memory impairment in D-galactose treated rats: role of anti-inflammatory pathways, Metab. Brain Dis. 37 (6) (2022) 1–16.
[50] D. Nieoczy, K. Socala, P. Wlaz, Anticonvulsant effect of pterostilbene and its influence on anxious and depressive behavior in mice fed with pentetrazole: behavioral, biochemical and molecular studies, Psychopharmacology 238 (2021) 3167–3181.
[51] M. Hosseini, S. Ghasemi, M. Moradzadeh, F. Beheshti, H.R. Sadeghnia, Beneficial effects of *Urtica dioica* on scopolamine-induced memory impairment in rats: protection against acetylcholinesterase activity and neuronal oxidative damage, Drug Chem. Toxicol. 42 (2) (2019) 167–175.
[52] G.S. Taiwe, F.C.O. Moto, E.R.M. Ayissi, G.T. Ngoupaye, J.S.K. Njapdounke, Effects of lyophilized aqueous extract of Feretia apodanthera Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice, Epilepsy Behav. 43 (2015) 100–108.
[53] D. Singh, R.K. Goel, Anticonvulsant mechanism of saponins fraction from adventitious roots of *Ficus religiosa*: possible modulation of GABAergics, calcium, and sodium channel functions, Revista Brasileira Farmacognosia 26 (5) (2016) 579–585.
[54] R.S. Fisher, E.B.W. Van, W. Blume, Epileptic seizures and epilepsy: definitions proposed by the international league against epilepsy (ILAE) and the international bureau for epilepsy (IBE), Epilepsia 46 (2010) 470–472.
[55] I. Uriarte-Pueyo, M.I. Calvo, Flavonoids as acetylcholinesterase inhibitors, Curr. Med. Chem. 18 (2011) 5289–5302.
[56] B. Yehezkel, GABA is a pioneering neurotransmitter for building the brain, Med. Sci. 23 (2007) 751–755.
[57] D. Singh, Cellular and molecular interactions of dietary flavonoids toward seizures suppression in epilepsy, in: Treatments, Nutraceuticals, Supplements, and Herbal Medicine in Neurological Disorders, Academic Press, 2023, pp. 305–325.
[58] L. Zhang, A.S. Ravipati, S.R. Koyyalamudi, S.C. Jeong, N. Reddy, P.T. Smith, J. Bartlett, K. Shanmugam, G. Münch, M.J. Wu, Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds, J. Agric. Food Chem. 59 (23) (2011) 12361–12367.
[59] S. Samina, Oxidative stress and psychological disorders, Curr. Neuropharmacol. 12 (2014) 140–147.
[60] N. Cichon, J. Saluk-Bijak, L. Gorniak, L. Przyslo, M. Bijak, Flavonoids as a natural enhancer of neuroplasticity-an overview of the mechanism of neurorestorative action, Antioxydants 9 (11) (2020) 1035.
[61] M. Sánchez, M. Galisteo, R. Vera, I.C. Villar, A. Zarzuelo, J. Tamargo, J. Duarte, Quercetin downregulates NADPH oxidase, increases eNOS activity, and prevents endothelial dysfunction in spontaneously hypertensive rats, J. Hypertens. 24 (2006) 75–84.
[62] N.J. Sucher, M.C. Carles, A pharmacological basis of herbal medicines for epilepsy, Epilepsy Behav. 52 (2015) 308–318.
[63] N. Ahmad, M.J. Al-Ghamdi, H.S. Alnajjad, B.B. Al Omar, M.F. Khan, Z.S. Almalki, A.A. Albassam, Z. Ullah, M.S. Khalid, K. Ashraf, A comparative Brain Toxico-Pharmacokinetics study of a developed Tannic Acid nanoparticles in the treatment of epilepsy, J. Drug Deliv. Sci. Technol. 76 (2022) 103772.
[64] A. Sun, X. Xu, J. Lin, X. Cui, R. Xu, Neuroprotection by saponins, Phytother Res. 29 (2) (2015) 187–200.
[65] G.A. Johnston, M. Chebib, R.K. Duke, S.P. Fernandez, J.R. Hanrahan, T. Hinton, K.N. Mewett, Herbal products and GABA receptors, Encyclopedia of Neuroscience 4 (2009) 1095–1101.
[66] M. Asif, Anticonvulsant potential of some traditional medicinal plants, Cell Med. 3 (4) (2014) 27–31, 2013.
[67] K.A. Kandeda, G.S. Taiwe, R.E. Ayissi, C. Moutchida, An aqueous extract of *Canarium schweinfurthii* attenuates seizures and potentiates sleep in mice: evidence for involvement of GABA Pathway, Biomed. Pharmacother. 142 (2021) 111973.