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Research article

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

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ABSTRACT

Ethnopharmacological relevance: Temporal lobe epilepsy is the most common form of drug-resistant epilepsy. Therefore, medicinal plants provide an alternative source for the discovery of new antiepileptic drugs.
Aim of the study: This study was aimed at investigating the antiepileptic- and anxiolytic-like effects of an aqueous extract of *Khaya senegalensis* (*K. senegalensis*) in kainate-treated rats.
Methods: Seventy-two rats received a single dose of kainate (12 mg/kg) intraperitoneally. Those that exhibited two hours of *status epilepticus* were selected and monitored for the first spontaneous seizure. Then, animals that developed seizures were divided into 6 groups of 8 rats each and treated twice daily for 14 days as follows: negative control group received *per os* (*p.o.*) distilled water (10 ml/kg); two positive control groups received either sodium valproate (300 mg/kg, *p.o.*) or phenobarbital (20 mg/kg, *p.o.*); and three test groups received different doses of the extract (50, 100, and 200 mg/kg, *p.o.*). In addition, a group of 8 normal rats (normal control group) received distilled water (10 ml/kg, *p.o.*). During the treatment period, the animals were video-monitored 12 h/day for behavioral seizures. At the end of the treatment period, animals were subjected to elevated plus-maze and open field tests. Thereafter, rats were euthanized for the analysis of γ-aminobutyric acid (GABA) concentration, oxidative stress status, and neuronal loss in the hippocampus.
Results: The aqueous extract of *K. senegalensis* significantly reduced spontaneous recurrent seizures (generalized tonic-clonic seizures) and anxiety-like behavior compared to the negative control group. These effects were more marked than those of sodium valproate or phenobarbital. Furthermore, the extract significantly increased GABA concentration, alleviated oxidative stress, and mitigated neuronal loss in the dentate gyrus of the hippocampus.
Conclusion: These findings suggest that the aqueous extract of *K. senegalensis* possesses antiepileptic- and anxiolytic-like effects. These effects were greater than those of sodium valproate or phenobarbital, standard antiepileptic drugs. Furthermore, these effects are accompanied by neuromodulatory and antioxidant activities that may be related to their behavioral effects. These data justify further studies to identify the bioactive molecules present in the extract for possible future therapeutic development and to unravel their mechanisms of action.

1. Introduction

Epilepsy is a chronic brain disorder characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences (Fisher et al., 2005; Beghi, 2020). The occurrence of at least two or more unprovoked seizures defines epilepsy (Fisher et al., 2014; Milligan, 2021; Pellinen et al., 2021). These seizures result from the excessive discharge of a group of

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neurons or, in some cases, all neurons of the brain (Falco-Walter et al., 2018; Fisher and Bonner, 2018). Around 50 million people worldwide suffer from epilepsy (Beghi, 2020; da Nobrega Marinho et al., 2022; Kwon et al., 2022), with the largest number living in low-income countries and sub-Saharan Africa (Preux and Druet-Cabanac, 2005; Owolabi et al., 2020). Cameroon seems to be the most affected country in Africa and the world, with an estimated prevalence between 5 and 136 ‰ (Preux and Druet-Cabanac, 2005; Prischich et al., 2008; Muhigwa et al., 2020). The highest prevalence was reported in the Sanaga-Mbam region, with 47.6% of the population having epilepsy. As a result, epilepsy represents the second most common reason for consultation in neurology (16%) in Cameroon, clearly making it a major public health concern (Mapoure et al., 2015; Angwafor et al., 2021; Morin et al., 2021). Of all types of epilepsy, temporal lobe epilepsy (TLE) accounts for 70–80% of adult epilepsies and is highly drug-resistant (Lévesque et Avoli, 2013; D'Alessio et al., 2020; Kandeda et al., 2021b; Kandeda et al., 2022).

TLE is a chronic brain disorder that is characterized by repeated seizures that originate in the temporal lobe of the brain (Lévesque et al., 2016), particularly starting in the dentate gyrus hippocampus and spreading to the CA1 and CA3 areas of the hippocampus (Lévesque et al., 2016; Bernhardt et al., 2019; Vrinda et al., 2019). At the molecular level, dysfunction in the γ-aminobutyric acid (GABA) signaling is primarily involved in the pathophysiology of epilepsy (Gale, 1992; Bozzi et al., 2018). However, accumulating studies revealed that the overproduction of reactive oxygen species (ROS) and free radicals is also involved in the pathogenesis of TLE (Frantseva et al., 2000; Shin et al., 2011; Kandeda et al., 2021a). Around half of people with TLE have frequent complaints about neuropsychiatric disorders such as anxiety and depression (Kimiskidis and Valeta, 2012; Gur-Ozmen et al., 2017; Lima et al., 2021). Their interictal anxiety behavior is associated with adverse outcomes such as drug resistance, poor surgical outcomes, and worse quality of life (Chapouthier and Venault, 2001; Vazquez and Devinsky, 2003; Kimiskidis and Valeta, 2012; Lima et al., 2021). Anxiety disorders in patients with TLE are due to multiple factors including multi-drug therapy, drug resistance, brain injury, and recurrent seizures (Chapouthier and Venault, 2001; Vazquez and Devinsky, 2003; Kimiskidis and Valeta, 2012; Lima et al., 2021). Also, anxiety disorders and TLE seem to share common neurobiology with the involvement amygdala and hippocampus (Chapouthier and Venault, 2001; Vazquez and Devinsky, 2003; Kimiskidis and Valeta, 2012; Lima et al., 2021). It is well known that the GABA inhibitory network plays an important role in the regulation of emotional responses in multiple pathological conditions. In particular, an impairment of neurotransmission mediated by GABAergic signaling is associated with the development of anxiety disorders (Vazquez and Devinsky, 2003; Beyenburg et al., 2005; Lima et al., 2021). In rodents, TLE can be modeled by systemic injection of kainate, an analog of glutamate (Ben-Ari, 1985; Lévesque and Avoli, 2013; Kandeda et al., 2022). In fact, kainate activates kainate/AMPA receptors through a form of permanent stimulation called excitotoxicity (Lévesque and Avoli, 2013; Wang et al., 2020; Landucci et al., 2021). This excitotoxicity leads to a reduction in seizure threshold and subsequent generation of spontaneous recurrent seizures (partial complex seizures with secondary generalization).

Despite the use of old antiepileptic drugs and the development of new ones (levetiracetam, topiramate, vigabatrin, tiagabine, lacosamide, and lamotrigine), therapy for TLE still faces many challenges: first, most antiepileptic drugs act on symptoms rather than on the underlying causes of the disease (Smith et al., 2007; Löscher and Brandt, 2010; Löscher, 2011); second, in people with brain injury, no treatment is available to prevent or inhibit the development of epilepsy (Löscher and Brandt, 2010; Ryther and Wong, 2012); and third, antiepileptic drugs are associated with multiple compelling side effects that limit their clinical use (Ortinski et al., 2004; de Kinderen et al., 2014). However, sodium valproate and phenobarbital are often first-line agents against TLE and other forms of epilepsy (Smith et al., 2007; Löscher and Brandt, 2010; Löscher, 2011; Marvin and Goldenberg, 2010; Ryther and Wong, 2012; Kandeda et al., 2017a). Based on these limits, the discovery and development of effective antiepileptic drugs with fewer side effects is of interest to these patients. Furthermore, in patients with anxiety disorders, there are no antiepileptic drugs that can effectively treat both seizures and anxiety disorders (Gower et al., 2003; Mula et al., 2007; Kaufman, 2011). Although preclinical research revealed anxiolytic effects for some antiepileptic drugs, the evidence for clinical effectiveness in epileptic patients is controversial (Gower et al., 2003; Schlaepfer and Nemeroff, 2012; Mula, 2018). Hence, antiepileptic drugs with clear anxiolytic effects are needed and traditional herbal medicine plants an interesting source for new drug candidates. In Cameroon and Asia, *K. senegalensis* (Desv.) A.Juss. (Meliaceae) (http://www.worldfloraonline.org) roots are used in traditional medicine to treat skin problems, jaundice, edema, neurological disorders, headaches, schizophrenia, epilepsy, and mental disorders (Maiga et al., 2005; Atawodi et al., 2014; Taiwe and Kuete, 2014). In Nigeria and West Africa, the stem bark and roots extracts are used to treat jaundice, syphilis, malaria, leprosy, dermatosis, epilepsy, and hookworm infections, while seeds and leaves are used to treat fever, headaches, mental illness (Audu-Peter et al., 2006; Sule et al., 2008; Taiwe and Kuete, 2014; Kinda et al., 2017). Pharmacological studies on the stem bark and roots of *K. senegalensis* have revealed anti-inflammatory (Thioune et al., 2000; Zhou et al., 2018), antioxidant, antibacterial, anthelmintic, anticancer, and immune-enhancing properties (Olayinka et al., 1992; Thioune et al., 2000; Audu-Peter et al., 2006; Kolawole et al., 2013; Atawodi et al., 2014). Furthermore, neuropharmacological studies of the aqueous extract of stem bark showed antiepileptic and sedative effects on seizures in mice (Ngo Bum et al., 2011). The main chemical groups present in the stem bark, leaves, and roots are alkaloids, tannins, phenols, saponins, sterols, triterpenes, cardiac glucosides, flavonoids, and reduced sugars (Kubmarawa et al., 2008; Makut et al., 2008; Atawodi et al., 2014). Gas chromatography-mass spectrometry (GS-MS) analysis of the aqueous extract of the stem bark mainly revealed the presence of n-Hexadecenoic acid, 9,12,15-octadecatrienoic acid, limonoids, khayandilobiride, oleic acid, and catechol (Aguoru et al., 2017; Allah et al., 2018; Zhou et al., 2018). Seneganolide A and khasenegasin G isolated from *K. senegalensis* showed *in vitro* neuroprotective effects against glutamate-induced injury (Olatunji et al., 2021). Toxicity studies with 21 days of treatment revealed that prolonged use of the aqueous extract of *K. senegalensis* stem bark may adversely affects vital organs (Kolawole et al., 2013; Onu et al., 2013; Muhammad et al., 2015). However, the adverse effects of this extract are only observed in high doses (Kolawole et al., 2013; Onu et al., 2013; Muhammad et al., 2015). In addition, based on the long tradition of use, traditional healers claim that the consumption of the aqueous extract of the roots does not induce as many adverse effects as the aqueous extract of the stem bark.

Given that this plant is empirically used to treat epilepsy, and epilepsy and anxiety share the same neurobiological basis, we hypothesized that the aqueous extract of the *K. senegalensis* roots may possess both antiepileptic- and anxiolytic effects. Therefore, this study aimed to investigate the antiepileptic and anxiolytic effects of an aqueous extract *K. senegalensis* roots, using the kainate model of TLE in rats. The effect of the extract was compared with sodium valproate and phenobarbital, standard antiepileptic drugs, often used as first-line agents against TLE. Further, potential neuromodulatory and antioxidant mechanisms were also explored.

2. Materials and methods

2.1. Plant collection and extract preparation

The roots of *K. senegalensis* were collected in the city of Ngaoundéré (Cameroon) in January 2018. A voucher specimen was deposited and authenticated at the National Herbarium of Cameroon (http://sweetgum.nybg.org/science/ih/herbarium-details/?irn=125449) under Number 856470/HNC. The plant name was also checked with plantlist.org. The protocol of the traditional healer was followed in this study. Briefly,

the roots of *K. senegalensis* were peeled off, cut, dried, and crushed. The obtained powder (50 g) was added to 750 ml of distilled water and then boiled for 20 min, cooled, and filtered through Whatman No. 1 paper. The mixture (742.5 ml) was then evaporated in an oven (55 °C), generating 7.4 g of dry extract, giving a yield of 14.8%. Thus, the concentration of the stock solution was 10 mg/ml. This stock solution was framed with a less concentrated solution (5 mg/ml) and a more concentrated solution (20 mg/ml). Knowing that all solutions are administered to animals in a volume of 10 ml/kg, so the above-mentioned concentrations were multiplied by 10 (Hull, 1995; Diehl et al., 2001; Turner et al., 2011). The extract of *K. senegalensis* was therefore administered *p.o.* at doses of 50, 100, and 200 mg/kg. The volume of administration used in the present study is based on previous laboratory studies (Hull, 1995; Diehl et al., 2001; Turner et al., 2011) or guidelines for the care of laboratory animals (IACUC standard procedure effective: approval on 29th September 2020 and University of Washington Institutional Animal Use and Care Committee Guideline: approval on 11th August 2019).

2.2. Animals and ethics

Male Wistar rats weighing approximately 200–250 g (3-month-old) were used. These rats were raised in the animal facilities of the Laboratory of Animal Physiology (University of Yaoundé I, Cameroon). They were kept in plastic cages (4 rats per cage) under ambient temperature (24–26 °C) and a cycle of natural light. Animals had free access to tap water and a standard diet. Prior authorization for all animal experiments was performed following the approval of the National Ethics Committee of Cameroon (Ref number FWA IRB00001954, 26th December 2005), which adopted the directive of the European Convention (Strasbourg, 18.III. 1986) for the protection of animals used for experimental studies (ETS-123) with particular attention to part III, articles 7, 8, and 9. The studies were also conducted according to American Veterinary Medical Association guidelines for the euthanasia of animals and ARRIVE 2.0 guidelines (https://arriveguidelines.org/arrive-guidelines). Blinding and randomization procedures during the studies were performed following the ARRIVE 2.0 guidelines. All efforts were made to minimize the number of rats and their suffering.

2.3. Chemicals and reagents

Kainate was purchased from Sigma Aldrich Co., St. Louis (USA), phenobarbital and sodium valproate from Sanofi-Aventis (France), diazepam from Roche (France), and ketamine hydrochloride from Hospira Inc (USA). Other chemicals and reagents used for biochemical tests were purchased from Sigma Aldrich Co., St. Louis (USA).

2.4. Induction of status-epilepticus and experimental design

Rats were divided into two groups and treated once as follows:

- a normal group of 8 rats received intraperitoneally (i.p.) distilled water (10 ml/kg);
- a group of 72 rats received kainate (12 mg/kg, i.p.).

Approximately 20 min after kainate injection, animals became hypoactive and displayed salivation, oro-facial movements, twitching of vibrissae, eye blinking, and yawning. The first generalized tonic-clonic seizure, which marked the beginning of limbic *status epilepticus* (SE), was observed 40–80 min after kainate injection. Indeed, it is well known that from stages 0–2, nonconvulsive seizures are observed after approximately 14 min from kainate administration (Lévesque and Avoli, 2013; Lévesque et al., 2016). These seizures are usually electroencephalogram seizures without behavioral signs (Lévesque and Avoli, 2013; Lévesque et al., 2016). The first stage 3 seizure occurred after another additional 23 min, while the first convulsive seizure (stage 4 or 5) required a time interval of approximately 1 h. Stage 5 seizures only occurred approximately 80 min after the injection of kainate. Animals that experienced 2 h of SE (prolonged seizures without returning to consciousness between episodes) were selected and injected with diazepam (10 mg/kg, i.p.) to reduce the severity of SE or mortality (Lévesque and Avoli, 2013; Lévesque et al., 2016). Immediately, the animals were then returned to their cages (3 per cage) and video-monitored for 10 h per day (from 8 h:00–18 h:00) until the appearance of the first spontaneous seizure (generalized tonic-clonic seizure) on day 30 after kainate-induced SE. The stage 5 seizures (generalized tonic-clonic seizures) were assessed during this period because they are the most frequent spontaneous recurrent seizures in this model (Williams et al., 2009; Lévesque and Avoli, 2013). At this point, the rats were randomized into six groups of 8 rats each. Then, they were treated twice daily for 14 days as follows:

- a negative control group named the KA + DW group received distilled water (10 ml/kg, *p.o.*);
- two positive control groups received sodium valproate 300 mg/kg, *p.o.*) named the KA + SVA group or phenobarbital (20 mg/kg, *p.o.*) named the KA + PhB group;
- test groups received the aqueous extract of *K. senegalensis* (50, 100, and 200 mg/kg, *p.o.*) named KA + KS50-200 groups.

In addition, a group of 8 normal rats (normal control group), named the DW + DW group, was added. This group was only treated with distilled water (10 ml/kg, *p.o.*).

During the treatment period, animals were video-monitored for 12 h per day (from 6 h:00–18 h:00) for the occurrence of spontaneous recurrent seizures (generalized tonic-clonic seizures or stage 5 seizures). The severity of seizures was assessed using the Racine scale (Phelan et al., 2015):

- stage 0: no reaction;
- stage 1: hyperactivity and clonus of vibrissae;
- stage 2: shaking of the head and myoclonic jerks;
- stage 3: unilateral clonus of the forelimbs;
- stage 4: rearing and bilateral clonus of the forelimbs;
- stage 5: tonic-clonic seizures with loss of righting reflex.

To allow animals time to recover from the chronic stress induced by the administration of treatments, the elevated plus-maze test for anxiety-like behavior was therefore performed 48 h after the last treatment (Lezak et al., 2017; Walf and Frye, 2007). At the end of the elevated plus-maze test, rats were left for 24 h before performing the open field test (Lezak et al., 2017; Walf and Frye, 2007). This was to reduce the impact of the stress induced by the elevated plus-maze test on the animals (Figure 1).

2.5. Elevated plus maze test

This test is based on the study of spontaneous behavior in animals (Rodgers and Dalvi, 1997). The experiment was carried out in the laboratory between 20 h and 24 h of the night because animals are hypoactive during this period of the day (Kandeda et al., 2022). The test consisted of placing each rat in the center of the device, facing an open arm for free exploration. The number of entries into each arm, the time spent in each arm, the number of rearings (the animal stands upright on its hind legs and rests on the edges of the experimental device), and the number of head dippings (animal looks under the experimental device) were recorded for 5 min. After each observation, the device was cleaned with ethanol (50°) before testing the next animal.

2.6. Open-field test

The open-field test is one of the most common tests for monitoring general motor activity, exploratory behavior, and anxiety-like behavior in rodents (Seibenhener and Michael, 2015; Kandeda et al., 2021a). The

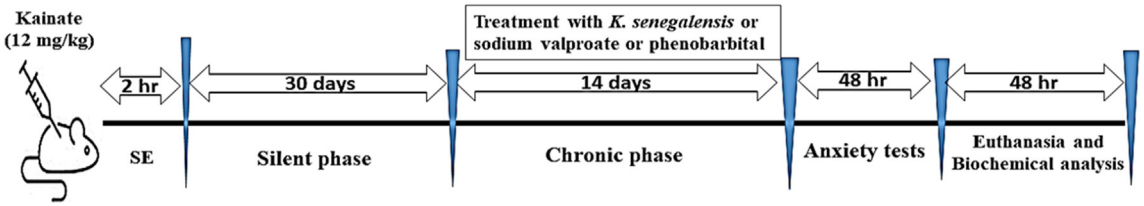

Figure 1. Schematic diagram of the experimental procedure. *K. senegalensis*: *Khaya senegalensis*; SE: *Status epilepticus*; hr: hour.

open field here was a wooden arena (278 × 236 × 300 mm). The normal behavior of rats is to seek protection from the periphery of the arena rather than vulnerability experienced at the center. Less anxious rats are expected to spend more time in the center rather than in the periphery. Rats were individually placed in the center of the arena and their behavior was monitored for 5 min. The photo beams recorded the following activities for 5 min: (i) the total time traveled in the inner zone; (ii) the total number of lines crossed in the central square (Kandeda et al., 2021a). All experiments were performed without the experimenter being present in the room.

2.7. Biochemical assays

2.7.1. Euthanasia and preparation of homogenates

Twenty-four hours after the completion of behavioral analysis, rats were euthanized under anesthesia with ketamine (50 mg/kg, i.p.) and diazepam (10 mg/kg, i.p.). The brain was then removed as quickly as possible (2–3 min), washed in cold 0.9% NaCl, pressed dry, and dissected to extract the hippocampus. For each animal, 0.1 g of hippocampus sample was added to 2 ml of Tris buffer (50 mM HCl; 150 mM KCl; pH 7.4) and homogenized with a Teflon glass homogenizer. The mixture was then centrifuged at 10,000 rpm for 15 min at room temperature (24–26 °C). The resulting supernatant was collected and stored at -20 °C for further biochemical analysis. To determine GABA transaminase (GABA-T) activity, 50 μg of the hippocampus sample was introduced into 500 μl of methanol (5%) and homogenized for 2 min at room temperature. The rest of the procedure was processed as below. During tissue preparation, ice-cold conditions were used.

2.7.2. Determination of GABA concentration

The amount of GABA in the hippocampus was assessed using the colorimetric technique described by Lowe et al. (1958). Ninhydrin reacts with succinic semialdehyde acid to form a colored complex. The absorbance of this complex is proportional to the amount of GABA in the homogenate. The working reagent was a mixture of 0.1 ml of 10% glacial trichloroacetic acid (TCA) and 0.2 ml of 0.14 M ninhydrin solution in a bicarbonate buffer solution (0.5 M; pH 9.9). The homogenate (100 μl) was then added to the working reagent. The mixture was incubated at 60 °C for 30 min. After cooling at room temperature, the solution was introduced into 5 ml of copper tartrate solution prepared from 0.16% disodium carbonate, 0.03% copper sulfate, and 0.033% tartaric acid. The whole was incubated at 25 °C for 10 min, and the color resulting from the reaction between ninhydrin and GABA was measured by a spectrophotometer at 570 nm. The concentration of GABA in the homogenate was determined using GABA standards (49, 99, 149, 199, 249, 299, 349, and 399 μg), each mixed with 0.3 mg of glutamate dissolved in 2 ml of 10% TCA. The concentration of GABA in the homogenate was expressed as μg/g of tissue.

2.7.3. Determination of glutamate decarboxylase (GAD) activity

The activity of GAD in the homogenate was determined according to Lowe et al. (1958). In the presence of glutamic acid, the increase in GABA concentration is proportional to GAD activity. The activity of GAD was determined using the amount of GABA during incubation with glutamic acid (Lowe et al., 1958). In 1 ml of homogenate and 1 ml of 0.05 M glutamic acid (neutralized at pH 6.3–6.7), 0.1 ml of pyridoxal-5-phosphate (25 pg) was added. The mixture was incubated at 37 °C for 30 min. After the incubation period, the reaction was stopped by heating at 100 °C for 10 min. The increase in GABA was monitored at 30 and 90 s with a spectrophotometer at 455 nm. The GAD activity was expressed as μg/min/mg of tissue.

2.7.4. Determination of GABA-T activity

The GABA-T activity was assessed using the colorimetric method (Nayak and Chatterjee, 2001). Succinic semialdehyde acid reacts with 3-methyl-2-benzothia-zolone-2-hydrazone in the presence of 12% $FeCl_3$ to form a colored complex (Nayak and Chatterjee, 2001). The coloration of the complex is proportional to the activity of GABA-T in the homogenate. Briefly, 15 μmol of GABA, 5 μmol of α-oxoglutarate, 0.1 ml of homogenate, and 10 μg of pyridoxal phosphate were introduced into test tubes, while in the blank tube, 0.2 ml of methanol (5%) was added. The volume of the mixture was made up to 3 ml with Tris-HCl buffer (50 mM HCl; 150 mM KCl; pH 7.4). The tubes were then incubated at 37 °C for 30 min for the reaction. The reaction was completed by adding 0.5 ml of 20%TCA. The amount of semialdehyde succinic acid generated during the incubation was read at 30 and 90 s by a spectrophotometer, at 610 nm against the blank. The GABA-T activity was expressed as μg/min/mg of tissue.

2.7.5. Determination of reduced glutathione (GSH) concentration

The concentration of GSH was determined according to the method described by Ellman (1959). The Ellman reagent (2,2-Dithio-5,5′-dinitrobenzoic acid) reacts with the SH groups of glutathione present in the homogenate. This reaction gives a yellow complex that absorbs at 412 nm (Ellman, 1959). For this assay, 1.5 ml of Ellman reagent were introduced into tubes already containing 100 μl of homogenate or 100 μl of Tris buffer (50 mM HCl; 150 mM KCl; pH 7.4) for the blank tube. The tubes were then vortexed and incubated for 1 h at room temperature. The absorbance was read by a spectrophotometer at 412 nm against the blank. The concentration of GSH was determined as mol/g of tissue.

2.7.6. Determination of malondialdehyde (MDA) concentration

The MDA concentration was determined by the method of Wilbur et al.(Wilbur et al., 1949). The presence of MDA leads to the formation of aldehydes. These compounds react with thiobarbituric acid (TBA) to form a pink complex that absorbs at 530 nm. The density of the pink coloration is proportional to the concentration of MDA in the homogenate (Wilbur et al., 1949). To perform this assay, 250 μl of homogenate were introduced into the test tubes, while 250 μl of Tris buffer (60 mM HCl; 150 mM KCl; pH 7.4) were introduced into the blank tube. To each tube, was added 125 μl of 20% trichloroacetic acid (TCA) and 250 μl of 0.67% TBA. The whole was incubated for 10 min at 90 °C, cooled at room temperature, and centrifuged at 4000 rpm for 15 min at room temperature. The supernatant absorbance was read by a spectrophotometer at 530 nm against the blank. The concentration of MDA was expressed as μmol/g of tissue.

2.7.7. Determination of superoxide dismutase (SOD) activity

The SOD activity was estimated following the method of Misra et Fridovish (Misra et Fridovish, 1972). The presence of SOD in the sample inhibits the oxidation of adrenaline to adrenochrome. The absorbance of adrenochrome is proportional to the activity of SOD. Briefly, the test

tubes contained 1666 µl of carbonate buffer (49 mM; pH 10.2) and 134 µl of homogenate, while the blank tubes contained 1800 µl of carbonate buffer. The reaction was started by adding 200 µl of 0.3 mM adrenaline solution. After the tubes were stirred for homogenization, the absorbance of the adrenochrome was read at 20 and 80 s at 480 nm by a spectrophotometer. The activity of SOD is defined in units of SOD/mg of tissue. The activity of SOD was expressed as U/min/mg of tissue.

2.7.8. Determination of nitrite concentration

The determination of nitric oxide (NO) was carried out according to the method of Griess (Foresi et al., 2016). The concentration of NO was expressed as mmol/g of tissue.

2.7.9. Determination of total protein concentration

The determination of total protein was carried out according to the method of Biuret (Baudet and Giddey, 1948). The concentration of total protein was expressed as mmol/g of tissue.

2.8. Histological analysis of the dentate gyrus of the hippocampus

Brain histology was processed by embedding 5-µm sections of tissue in the paraffin followed by hematoxylin-eosin staining. In this study, three sections per rat of the dentate gyrus were examined. The analysis was performed using Zeiss microscope equipment (Hallbermoos, Germany) connected to a computer. The obtained images were analyzed with Image J software (version 1.52) to quantify the number of surviving neurons per μm^2. Surviving neurons were differentiated from glial cells in terms of structure and staining; neurons have axons and dendrites that serve to transfer electrical signals between other nerve cells. In contrast, glial cells lack axons and dendrites.

2.9. Statistics

The statistical analysis of the values was performed using GraphPad Prism version 8.01 and Microsoft Office Excel 2013 version 15.0.4420.1017. Results were expressed as average ±standard error of the mean (SEM) or as percentages. Values were compared using a one-way or two-way analysis of variance (ANOVA). The percentage of protection was compared using Fisher's exact test (two-tails). In case of difference between groups, the Tukey multiple comparison *post-hoc* test was used to separate them. Difference in values was considered significant at $p < 0.05$.

3. Results

3.1. Effect of the aqueous extract of K. senegalensis on the incidence, number, and duration of spontaneous recurrent seizures

During the chronic phase of epilepsy, kainate-induced SE caused (Fisher exact test, $p < 0.001$) spontaneous recurrent seizures (generalized tonic-clonic seizures or stage 5 seizures) in the KA + DW group compared to the DW + DW group (Figure 2A). During 14 days of treatment, the

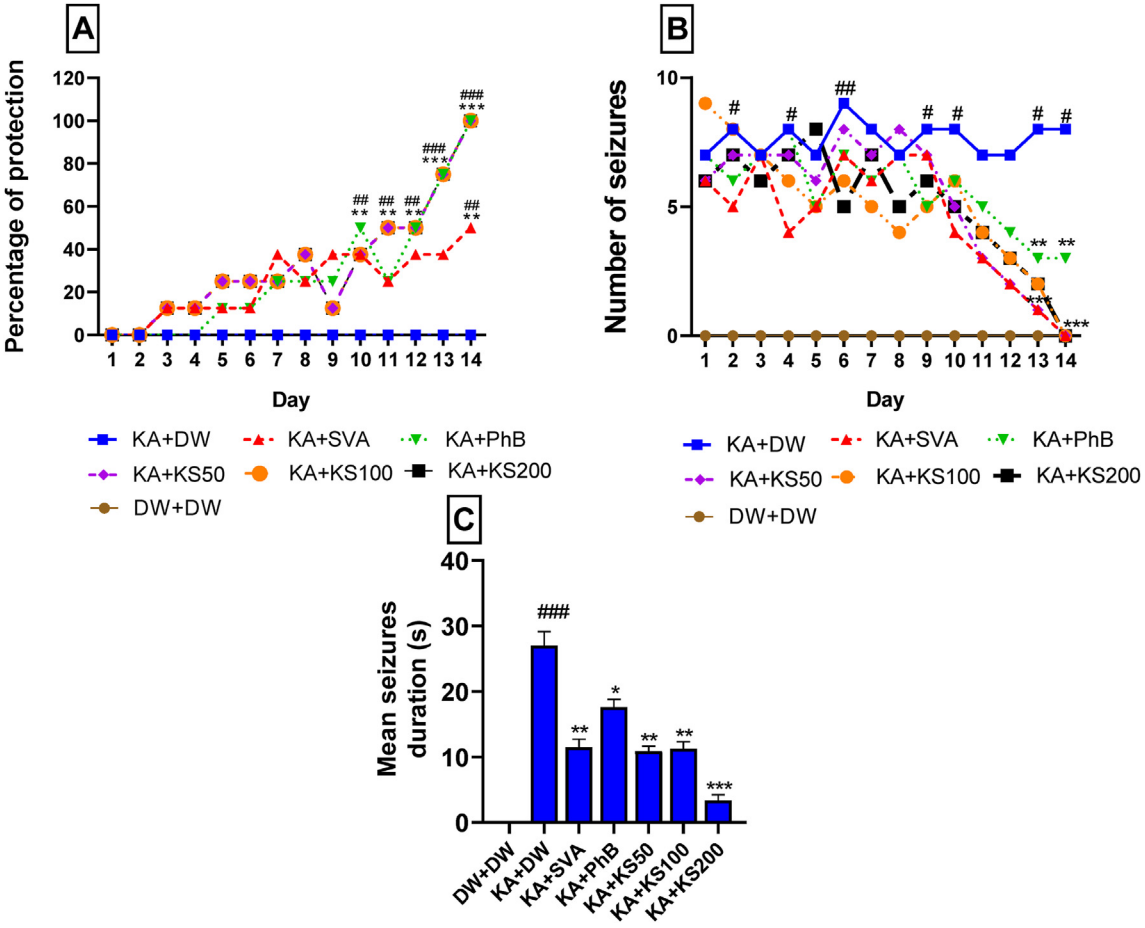

Figure 2. Effect of the aqueous extract of *K. senegalensis* on the incidence (A), number (B), and mean duration (C) of spontaneous recurrent seizures. Each bar represents the average ±SEM; n = 8. #p < 0.05, ##p < 0.01, ###p < 0.001 *vs* normal control. *p < 0.05, **p < 0.01, ***p < 0.001 *vs* negative control. DW: Distilled water (10 ml/kg); KA: Kainate (12 mg/kg); SVA: Sodium valproate (300 mg/kg); PhB: Phenobarbital (20 mg/kg); KS50-200: Aqueous extract of *K. senegalensis* at respective doses of 50, 100, and 200 mg/kg. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KA + KS50-200: Test groups treated with *K. senegalensis* extract 50, 100, and 200 mg/kg.

extract gradually protected animals against spontaneous recurrent seizures. However, the extract at all doses protected rats against 100% ($p < 0.001$) of rats on the 14th day of treatment (Figure 2A). On the 13th and 14th days of treatment, sodium valproate and phenobarbital protected 50% ($p < 0.01$) and 100% ($p < 0.001$) of the animals, respectively (Figure 2A).

Two-way ANOVA revealed significant inter-group differences in the number of spontaneous recurrent seizures [$F(6, 78) = 41.07$, $p < 0.0001$]. During 14 days of treatment, kainate-induced SE caused an increase in the number of spontaneous recurrent seizures in the KA + DW group compared to the DW + DW group. However, the highest increase was observed on the 6th day (9 seizures, $p < 0.01$) (Figure 2B). The extract at all doses reduced this number to 0 ($p < 0.001$) on the 14th day of treatment. Similarly, sodium valproate decreased this number to 0 ($p < 0.001$), while phenobarbital decreased this number to 3 ($p < 0.01$), on the 14th day (Figure 2B).

One-way ANOVA revealed significant inter-group differences in the mean duration of spontaneous recurrent seizures [$F(6, 49) = 53.83$, $p < 0.0001$]. During 14 days of treatment, kainate-induced SE led to an increase in the mean duration of spontaneous recurrent seizures to 27.00 ± 2.20 s ($p < 0.001$) in the KA + DW group compared to the DW + DW group (Figure 2C). The extract at all doses decreased this parameter. However, the extract (200 mg/kg) markedly decreased this time (3.38 ± 0.89 s, $p < 0.001$) (Figure 2C). Sodium valproate and phenobarbital also decreased this time to 11.25 ± 1.10 s ($p < 0.001$) and 17.63 ± 1.19 s ($p < 0.05$), respectively (Figure 2C).

3.2. Effect of the aqueous extract of K. senegalensis on the anxiety-like behavior in the elevated plus maze

One-way ANOVA revealed significant inter-group differences in the number of entries in open or closed arms [$F(6, 49) = 15.85$, $p < 0.0001$]. Kainate-induced SE caused a decrease in the percentage of entries in the open arms to 12.62 ± 0.56% ($p < 0.001$) in the KA + DW group compared to the DW + DW group (Figure 3A). The extract dose-dependently increased the percentage of entries in these arms. However, a dose of 200 mg/kg of the extract showed the highest increase in this percentage (47.08 ± 0.34%, $p < 0.001$) (Figure 3A). Sodium valproate and phenobarbital induced a nonsignificant variation (Figure 3A).

Kainate-induced SE led to an increase in the percentage of entries in the closed arms to 87.38 ± 0.21% ($p < 0.001$) in the KA + DW group compared to the DW + DW group (Figure 3A). The extract dose-dependently decreased this number. However, the highest dose of the extract showed the greatest reduction in this number (52.92 ± 0.49%, $p < 0.001$) (Figure 3A). Sodium valproate and phenobarbital induced a nonsignificant decrease (Figure 3A).

One-way ANOVA revealed significant inter-group differences in the percentage of time spent in open or closed arms [$F(6, 49) = 66.02$, $p < 0.0001$]. Kainate-induced SE induced a decrease in the percentage of time spent in the open arms to 5.21 ± 1.21% in the KA + DW group compared to the DW + DW group (Figure 3B). The extract at all doses induced a significant increase. However, the extract at a dose of 200 mg/kg remarkably increased this percentage (91.31 ± 0.29%, $p < 0.001$) (Figure 3B). Sodium valproate and phenobarbital also increased this percentage to 35.10 ± 6.51% ($p < 0.001$) and 40.55 ± 4.12% ($p < 0.001$), respectively (Figure 3B).

Kainate-induced SE caused an increase in the percentage of time spent in the closed arms to 98.34 ± 0.32% ($p < 0.05$) in the KA + DW group compared to the DW + DW group (Figure 3B). The extract at all doses significantly decreased this percentage. However, the extract at a dose of 200 mg/kg induced the highest reduction in this percentage (8.69 ± 0.85%, $p < 0.001$) (Figure 3B). Sodium valproate and phenobarbital equally decreased this percentage to 35.10 ± 0.66% ($p < 0.001$) and 40.55 ± 3.96% ($p < 0.001$), respectively (Figure 3B).

3.3. Effect of the aqueous extract of K. senegalensis on the anxiety behavior in the open field

One-way ANOVA revealed significant inter-group differences in the number of line crossings in the open field [$F(6, 20) = 29.98$, $p < 0.0001$]. Kainate-induced SE caused a decrease in the number of line crossings by 42.31% ($p < 0.05$) in the KA + DW group compared to the DW + DW group (Figure 4A). The extract at all doses led to an increase in the number of crossings, with a two-fold ($p < 0.001$) increase of this number at doses of 50 and 200 mg/kg. Likewise, sodium valproate and phenobarbital increased this number by 100% ($p < 0.01$) (Figure 4A).

One-way ANOVA revealed significant inter-group differences in the time spent in the center of the open field [$F(6, 20) = 13.17$, $p < 0.0001$]. Compared to the DW + DW group, kainate-induced SE caused a reduction in the time spent in the center by 74.42% ($p < 0.001$) in the KA + DW group (Figure 4B). The extract at all doses induced an increase in this time, with a two-fold ($p < 0.001$) increase at doses of 50 and 200 mg/kg. Sodium valproate and phenobarbital induced a similar two-fold ($p < 0.001$) increase (Figure 4B).

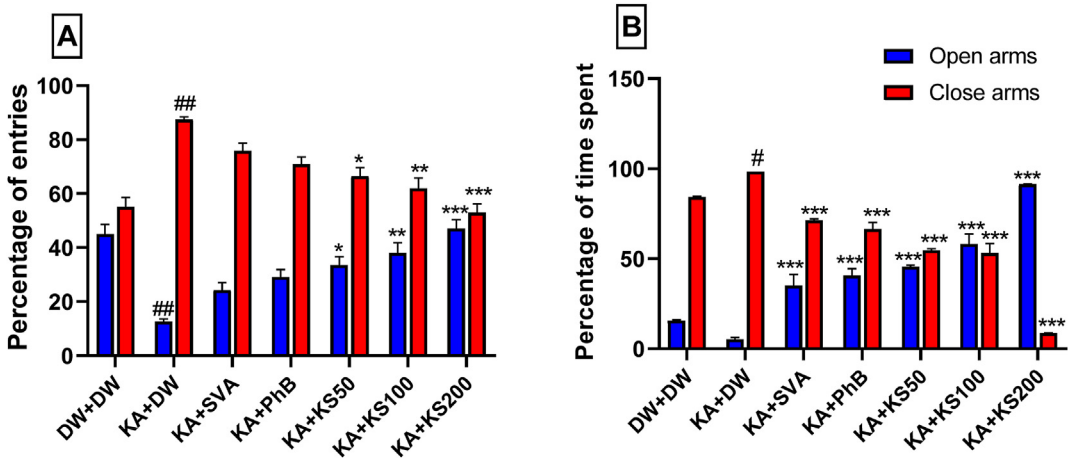

Figure 3. Effect of the aqueous extract of *K. senegalensis* on the anxiety behavior in the elevated plus maze. (A): Percentage of entries, (B): Percentage of time spent. Each bar represents the average ±SEM; n = 8. #$p < 0.05$, ##$p < 0.01$ *vs* normal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs* negative control group. DW: Distilled water (10 ml/kg); KA: Kainate (12 mg/kg); SVA: Sodium valproate (300 mg/kg); PhB: Phenobarbital (20 mg/kg); KS50-200: Aqueous extract of *K. senegalensis* at respective doses of 50, 100 and 200 mg/kg. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KA + KS50-200: Test groups treated with *K. senegalensis* extract 50, 100, and 200 mg/kg.

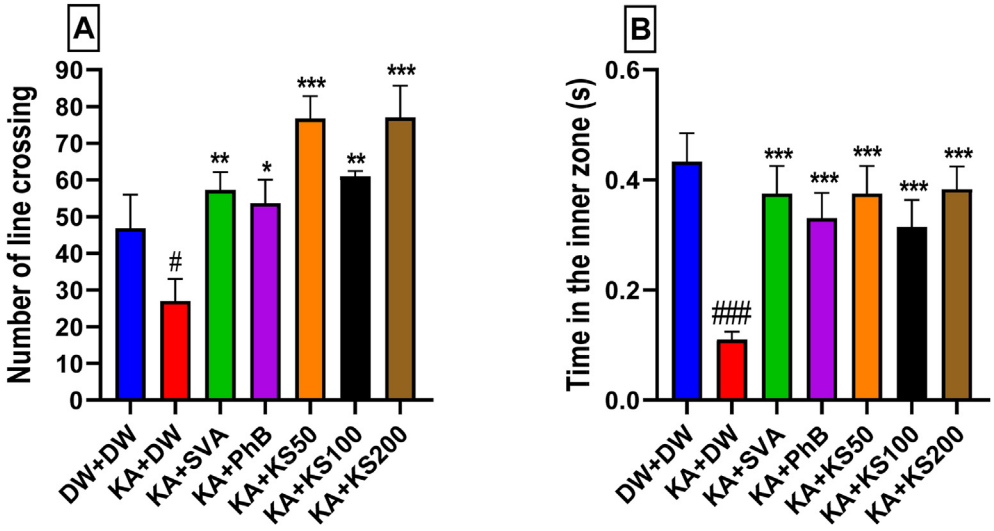

Figure 4. Effect of the aqueous extract of *K. senegalensis* on the anxiety behavior in the open field. (A): Number of line crossing, (B): Time spent in the inner zone. Each bar represents the average ±SEM; n = 8. #p < 0.05, ###p < 0.001 *vs* DW + DW group. *p < 0.05, **p < 0.01, ***p < 0.001 *vs* KA + DW group. DW: Distilled water (10 ml/kg); KA: Kainate (12 mg/kg); SVA: Sodium valproate (300 mg/kg); PhB: Phenobarbital (20 mg/kg); KS50-200: Aqueous extract of *K. senegalensis* at respective doses of 50, 100 and 200 mg/kg. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KA + KS50-200: Test groups treated with *K. senegalensis* extract 50, 100, and 200 mg/kg.

3.4. Effect of the aqueous extract of K. senegalensis on the GABA metabolism in the hippocampus

One-way ANOVA revealed significant inter-group differences in the GABA concentration [F (6, 28) = 748.3, p < 0.0001]. Kainate-induced SE caused a decrease in the GABA concentration by 64.14% (p < 0.001) in the KA + DW group compared to the DW + DW group (Figure 5A). The extract at all doses increased the concentration of GABA. The extract (50 mg/kg) induced the highest increase in this concentration (232.43%, p < 0.001) (Figure 5A). Sodium valproate induced a smaller increase, by 52.83% (p < 0.001) (Figure 5A).

One-way ANOVA revealed significant inter-group differences in the GAD activity [F (6, 28) = 101.7, p < 0.0001]. Kainate-induced resulted in a decrease in the GAD activity by 50.60% (p < 0.001) SE in the KA + DW group compared to the DW + DW group (Figure 5B). The extract (50 mg/kg) induced the highest increase in this activity (77.50%, p < 0.001) (Figure 5B). Sodium valproate induced a similar increase, reaching 78.34% (p < 0.001) (Figure 5B).

One-way ANOVA revealed significant inter-group differences in the GABA-T activity [F (6, 28) = 8.851, p < 0.0001]. Kainate-induced SE led to an increase in the GABA-T activity by 56.11% (p < 0.001) in the KA + DW group compared to the DW + DW group (Figure 5C). The extract at a dose of 200 mg/kg markedly induced a decrease in this activity (83.72%, p < 0.001) (Figure 5C). Sodium valproate induced a decrease in this activity by 64.36% (p < 0.001) (Figure 5C).

3.5. Effect of the aqueous extract of K. senegalensis on some oxidative stress parameters in the hippocampus

One-way ANOVA revealed significant inter-group differences in the concentration of GSH [F (6, 28) = 225, p < 0.0001]. Kainate-induced SE led to a decrease in the GSH concentration by 43.16% (p < 0.001) in the KA + DW group, compared to the DW + DW group (Table 1). The extract (50 mg/kg) led to a marked increase in this concentration (52.76%, p < 0.001) (Table 1). Sodium valproate and phenobarbital induced an increase in this concentration by 55.91% (p < 0.001) and 41.14% (p < 0.001), respectively (Table 1).

One-way ANOVA revealed significant inter-group differences in the concentration of MDA [F (6, 28) = 79.34, p < 0.0001]. Kainate-induced SE resulted in an increase in the MDA concentration by 77.92% (p < 0.001) in the KA + DW group compared to the DW + DW group (Table 1). The extract at the highest dose markedly decreased this concentration (57.48%, p < 0.001). Sodium valproate and phenobarbital failed to induce a decrease in this concentration (Table 1).

One-way ANOVA revealed significant inter-group differences in the activity of SOD [F (6, 28) = 134.6, p < 0.0001]. Kainate-induced SE resulted in an increase in the SOD activity by 912 ± 0.2 U/min/μg (p < 0.001) in the KA + DW group compared to the DW + DW group (Table 1). The extract at a dose of 200 mg/kg remarkably decreased this activity by 81.61% (p < 0.001) (Table 1). Sodium valproate and phenobarbital led to a decrease in this activity by 82.57% (p < 0.001) and 40.43% (p < 0.001), respectively (Table 1).

One-way ANOVA revealed significant inter-group differences in the concentration of NO [F (6, 28) = 1944, p < 0.0001]. Kainate-induced SE caused a decrease in the concentration of NO by 63.00% (p < 0.001) in the KA + DW group compared to the DW + DW group (Table 1). The extract at a dose of 200 mg/kg markedly increased this concentration by 63.33% (p < 0.001) (Table 1). Sodium valproate and phenobarbital decreased this concentration by 18.64% (p < 0.001) and 28.68% (p < 0.001), respectively (Table 1).

One-way ANOVA revealed significant inter-group differences in the concentration of total protein [F (6, 28) = 243.2, p < 0.0001]. Kainate-induced SE led to a decrease in total protein concentration by 84.02% (p < 0.001) in the KA + DW group compared to the DW + DW group (Table 1). The extract (200 mg/kg) induced a great increase in this concentration (80.34%, p < 0.001) (Table 1). Sodium valproate and phenobarbital increased this concentration by 61.01% (p < 0.001) and 66.17% (p < 0.001), respectively (Table 1).

3.6. Effect of the aqueous extract of K. senegalensis on the morphology and density of neurons in the dentate gyrus of hippocampus

The micro-architecture of the dentate gyrus in the DW + DW group showed a regular thickness (Figure 6A, image) and normal neuronal density (Figure 6B, numerical values). In contrast, in the KA + DW group, a reduced neuronal density, perivascular edema, and granulovascular degeneration were observed (Figure 6A). These alterations were confirmed by a reduction in the number of surviving neurons [F (6, 28) = 202.7, p < 0.05] (Figure 6B). In animals treated with the extract (100 and 200 mg/kg), the dentate gyrus presented a regular thickness (Figure 6A). Also, these doses reduced (p < 0.001) neuronal loss (Figure 6B). These effects were similar to those observed with sodium valproate, an antiepileptic drug with a neuroprotective property (Figures 6A and B).

4. Discussion

This study aimed to evaluate the antiepileptic- and anxiolytic-like effects of an aqueous extract of *K. senegalensis* on kainate-induced TLE

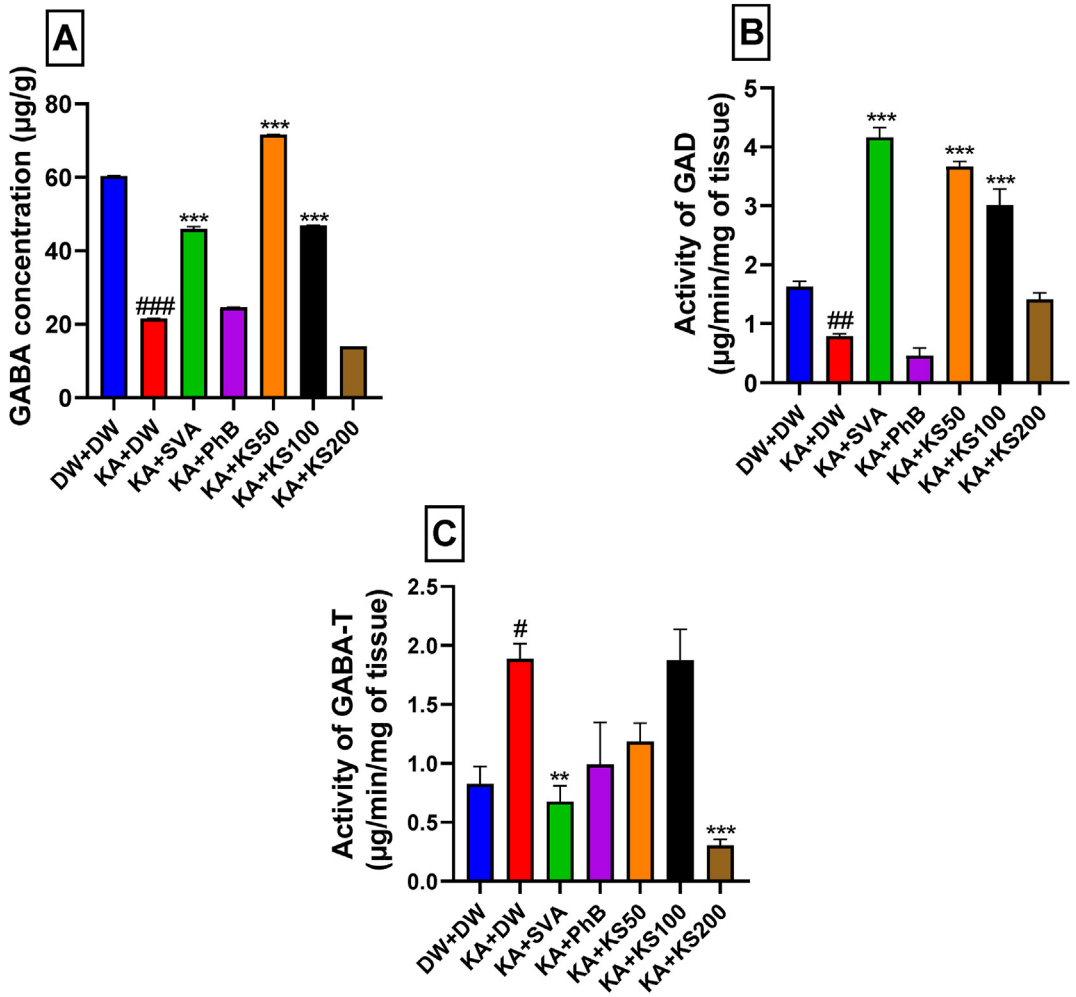

Figure 5. Effect of the aqueous extract of *K. senegalensis* on GABA metabolism. (A): GABA concentration, (B): GAD activity, and (C): GABA-T activity in the hippocampus. Each bar represents the average ±SEM; n = 8. #p < 0.05, bp < 0.01, ###p < 0.001 *vs* DW + DW group. **p < 0.01, ***p < 0.001 *vs* KA + DW group. DW: Distilled water (10 ml/kg); KA: Kainate (12 mg/kg); SVA: Sodium valproate (300 mg/kg); PhB: Phenobarbital (20 mg/kg); KS50-200: Aqueous extract of *K. senegalensis* at respective doses of 50, 100, and 200 mg/kg. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KA + KS50-200: Test groups treated with *K. senegalensis* extract 50, 100, and 200 mg/kg.

in rats. In adult humans, TLE is the most common form of epilepsy (Engel, 2013) and is characterized by spontaneous and recurrent seizures, often refractory to antiepileptic drugs (Engel, 2013). Although no animal model exhibits all the features of TLE, some of them have been widely used because of their similarity to the human form of epilepsy (Lévesque and Avoli, 2013; Lévesque et al., 2016). One of these is the kainate model of TLE, which was discovered by Ben-Ari (1985). In their work, these researchers demonstrated that injection of KA (an agonist of ionotropic KA receptors and an analog of L-glutamate) into the amygdala leads to spontaneous seizures and brain damage similar to those seen in people with TLE (Lévesque and Avoli, 2013). The kainate model of TLE is a valid tool for understanding the pharmacological, cellular, and molecular mechanisms underlying seizures and epileptogenesis (Lévesque and Avoli, 2013). This model is characterized by an initial precipitating injury (i.e., SE occurring approximately in 94% of animals) followed by a seizure-free latency period (epileptogenesis). Spontaneous recurrent seizures occur a few weeks or months after the SE-inducing insult (Williams et al., 2009; Wu et al., 2009). In the present study, kainate-induced SE led to spontaneous recurrent seizures (generalized tonic-clonic seizures or stage 5 seizures) in all rats of the KA + DW group compared to those of the DW + DW group. These rats also exhibited a high number and duration of seizures. It is well established that after systemic administration of kainate (12–15 mg/kg, i.p.), SE develops between 20–90 min and lasts for approximately 2–9 h (if seizures are not stopped with an injection of an anesthetic), according to multiple experiments on rats (Lévesque and Avoli, 2013; Rusina et al., 2021). In 60–80% of animals that survive after kainate-induced SE, non-convulsive seizures occur 10–30 days after SE (Lévesque and Avoli, 2013; Rusina et al., 2021). During this period, SE-induced excitotoxicity leads to multiple molecular and cellular changes, mainly in the hippocampus (mossy fibers sprouting, granular cells dispersion, decrease in the expression of $GABA_A$ receptors, increase in the expression of NMDA or kainate receptors, neuronal loss, neuroinflammation, oxidative stress, astrogliosis, and decrease in the concentration of extracellular K+) (Hsieh et al., 1999; Lévesque and Avoli, 2013; Van Nieuwenhuyse et al., 2015; Rusina et al., 2021). Altogether, these changes result in a reduced seizure threshold and generation of spontaneous recurrent seizures, which mark the beginning of the chronic phase of epilepsy (Hsieh et al., 1999; Lévesque and Avoli, 2013; Van Nieuwenhuyse et al., 2015; Rusina et al., 2021). On the 14th day of treatment after the occurrence of the first spontaneous seizures, the extract at all doses completely protected 100% of rats against spontaneous recurrent seizures compared to the rats of the KA + DW group. Since recurrent spontaneous seizures induced by kainate-induced SE are complex partial seizures with secondary generalization, and these seizures are attenuated or inhibited by some antiepileptic drugs such as sodium valproate, carbamazepine, levetiracetam, phenobarbital (Hsieh

Table 1. Effect of *K. senegalensis* extract on some oxidative stress parameters in the hippocampus.

Treatments	GSH (mol/g)	MDA (µmol/g)	Total protein (mmol/g)	NO (mmol/g)	SOD (U/min/mg)
DW + DW	13.97 ± 0.05	8.48 ± 0.32	1.44 ± 0.41	182.00 ± 1.11	8.23 ± 0.77
KA + DW	7.94 ± 0.02###	38.41 ± 1.20###	0.23 ± 0.03###	67.67 ± 2.49###	912.95 ± 0.18###
KA + SVA	12.38 ± 0.26***	38.36 ± 1.28	0.59 ± 0.03***	83.18 ± 4.35***	159.11 ± 10.04***
KA + PhB	13.49 ± 0.23	35.53 ± 0.75	0.68 ± 0.04***	94.89 ± 3.30***	543.78 ± 67.24
KA + KS50	16.76 ± 0.23***	21.25 ± 0.77***	0.58 ± 0.03***	106.30 ± 2.52***	207.36 ± 18.48***
KA + KS100	14.11 ± 0.22***	22.56 ± 0.95***	0.78 ± 0.05***	130.80 ± 2.74***	175.80 ± 11.04***
KA + KS200	15.11 ± 0.05***	16.33 ± 2.63***	1.17 ± 0.05***	184.54 ± 1.77***	167.87 ± 6.44***

Each value represents the average ±SEM; n = 8. ###p < 0.001 *vs* DW + DW group. ***p < 0.001 *vs* KA + DW group. DW: Distilled water (10 ml/kg); KA: Kainate (12 mg/kg); SVA: Sodium valproate (300 mg/kg); PhB: Phenobarbital (20 mg/kg); KS50-200: Aqueous extract of *K. senegalensis* at respective doses of 50, 100, and 200 mg/kg. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KA + KS50-200: Test groups treated with *K. senegalensis* extract at doses 50, 100, and 200 mg/kg; GSH: Reduced glutathione; MDA: Malondialdehyde; NO: Nitric oxide; SOD: Superoxide dismutase.

et al., 1999; Smith et al., 2007; Löscher and Brandt, 2010; Löscher, 2011; Wu et al., 2009; Lévesque and Avoli, 2013). These results suggest therefore that the aqueous extract of *K. senegalensis* possesses an antiepileptic-like effect. This effect could be mediated through the interaction of the extract with the above-mentioned mechanisms. Thus, further study needs to be performed to determine the exact mechanisms of the extract, using *in vivo* or *in vitro* experimental models of refractory epilepsy These results also suggest an effect of the extract on the underlying causes of seizures. Thus, further studies are needed to find out whether the extract acts on the causes of the disease rather than on the symptoms. In addition, the effect of the extract was greater than that of sodium valproate or phenobarbital, standard antiepileptic drugs used against TLE, and GABA-activating drugs (Löscher and Brandt, 2010; Löscher, 2011; Marvin and Goldenberg, 2010; Ryther and Wong, 2012; Kandeda et al., 2017a; Romoli et al., 2019). The effect of phenobarbital was lesser than that of sodium valproate possibly because phenobarbital is more susceptible to dependence (Löscher and Brandt, 2010; Löscher, 2011; Marvin and Goldenberg, 2010). All these findings equally suggest a possible interaction of the extract with GABA neurotransmission. Recent studies on the antiepileptic effect of the aqueous extract of *K. senegalensis* stem bark support the presence in the extract of bioactive molecules with antiepileptic-like properties (Ngo Bum et al., 2011; Kolawole et al., 2013). Thus, these findings also provide scientific evidence for the empirical use of the aqueous extract of *K. senegalensis* against epilepsy in Africa (Taïwe and Kuete, 2014; Kinda et al., 2017).

Anxiety is the most common neuropsychiatric complaint in patients with TLE (Kimiskidis and Valeta, 2012; Gur-Ozmen et al., 2017; Lima et al., 2021). Indeed, about half of people with TLE often complain about neuropsychiatric disorders such as anxiety and depression (Kimiskidis and Valeta, 2012; Gur-Ozmen et al., 2017; Lima et al., 2021). Although

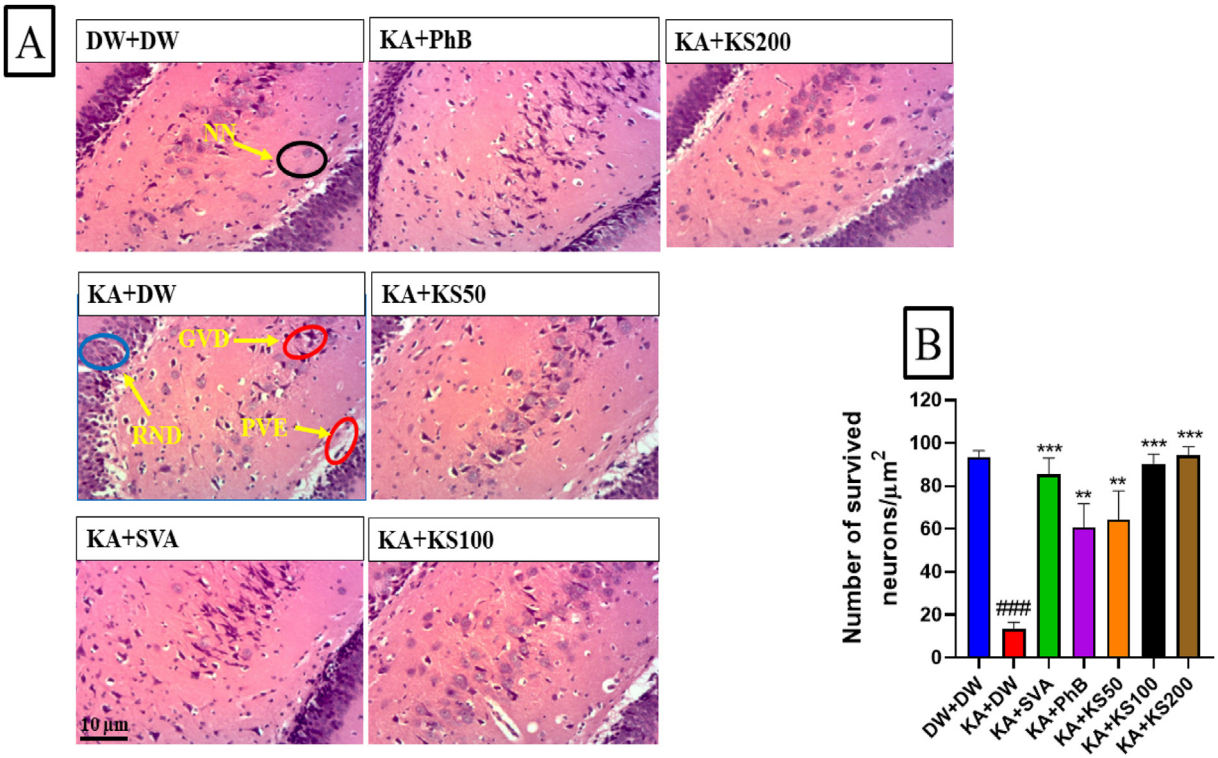

Figure 6. Effect of the aqueous extract of *K. senegalensis* on the histology of the dentate gyrus (hippocampus) following staining with hematoxylin and eosin (×200). (A): Microarchitecture (25µm-scale), (B): Number of surviving neurons per µm^2. Each value represents the average ±SEM; n = 8. ###p < 0.001 *vs* DW + DW group. **p < 0.01, ***p < 0.001 *vs* KA + DW group. DW + DW: Normal control group; KA + DW: Negative control group; KA + SVA: Positive control group with sodium valproate; KA + PhB: Positive control group with phenobarbital; KS50-200: Test groups treated with *K. senegalensis* extract (50, 100, and 200 mg/kg); NN: Normal neuron; PVE: Perivascular edema; GVD: Granovascular degeneration; RND: Reduced neuronal density.

some antiepileptic drugs revealed controversial anxiolytic effects in patients with epilepsy (Gower et al., 2003; Higgins et al., 2009), an antiepileptic drug that effectively and consistently targets both seizures and anxiety pathogenesis is needed (Gower et al., 2003; Higgins et al., 2009). In rodents, anxiety-like behaviors are tested using several paradigms such as elevated plus maze and open field (Rodgers and Dalvi, 1997; Umpierre et al., 2014; Smolensky et al., 2019). The elevated plus maze and open field tests are therefore used to assess the sensitivity of medications to generalized anxiety (Rodgers and Dalvi, 1997; Seibenhener and Michael, 2015; Kandeda et al., 2021a), a common form of anxiety in patients with TLE (Özyurt et al., 2015; Nogueira et al., 2017; Kandeda et al., 2022). In the present study, 48 h following the end of treatments, animals of the KA + DW group exhibited generalized anxiety-like behavior compared to the DW + DW group. In the elevated plus-maze test, this behavior was characterized by an increase in the number of entries and time spent in closed arms. In these animals, there was also a decrease in the number of entries and time spent in the open arms compared to the DW + DW group. These observations in the elevated plus-maze test were confirmed in the open field test with a decrease in the number of crossings and time spent in the inner zone. Experimentally, systemic injection of kainate is subsequently associated with the development of lesions in the striatum, cortex, entorhinal, piriform cortex, subiculum hippocampus, and amygdala complex (Lévesque and Avoli, 2013; Lévesque et al., 2016). However, the amygdala complex, one of the components of the temporal lobe, is involved in the production of emotion, stress, and fear (Aroniadou-Anderjaska et al., 2008). Therefore, an injury in this region is associated with a change in emotional behavior or the development of anxious behavior during epileptogenesis or chronic epilepsy (Lévesque and Avoli, 2013; Lévesque et al., 2016). Indeed, this brain region displays a high expression of kainate receptors on the GABA interneurons where they block the release of GABA (Ryazantseva et al., 2020; Negrete-Díaz et al., 2021). Furthermore, during the chronic phase of epilepsy, a higher frequency of seizures is associated with anxiety behavior and amygdala lesions (Arulsamy and Shaikh, 2016). In the elevated plus-maze test, the extract at the dose of 200 mg/kg induced the highest decrease in the number of entries and time spent in closed arms compared to the KA + DW group. In these animals, the extract also induced an increase in the number of entries and time spent in the open arms. These results in the elevated plus maze test were confirmed in the open field test with an increase in the number of crossings and time spent in the inner zone. These results indicate an anxiolytic-like effect of the extract of *K. senegalensis* against generalized anxiety (Moto et al., 2018; Kandeda et al., 2022). This effect could be mediated by an interaction of the extract with GABAergic signaling or downregulation of glutamate neurotransmission in the amygdala (Ryazantseva et al., 2020; Negrete--Díaz et al., 2021). The effect of the extract could also result from a protective activity against the loss of GABAergic interneurons in the amygdala (Mishra et al., 2015). Metabolomic studies are needed to identify possible pathways through which the extract exerts its anxiolytic-like effect.

In an attempt to find possible mechanisms of action involved in the antiepileptic- and anxiolytic-like effects of the extract, the involvement of the GABA pathway was explored since this neurotransmitter plays a major role in epilepsy and anxiety (Gale, 1992; Nayak and Chatterjee, 2001). Compelling evidence has shown that kainate-induced SE results in epileptogenesis, which is associated with decreased $GABA_A$ receptors expression, increased glutamate concentration, and decreased GABA concentration in the hippocampus (Krespan et al., 1982; Ueda et al., 2001; Sperk et al., 2003). These changes imply an alteration of GABA-T and GAD activities. Our data showed that kainate-induced SE led to a decrease in GABA and GAD levels in the KA + DW group compared to the DW + DW group, whereas it led to an increase in GABA-T activity, as previously shown by Ortiz et al. (2001) and Sperk et al. (2003). According to these authors, this decrease is an obvious manifestation of a loss of GABAergic interneurons and a decrease in $GABA_A$ receptors expression in the hippocampus. The extract of *K. senegalensis* (50 and 100 mg/kg) significantly increased the concentration of GABA and suggested interference with the GABA pathway. These effects of the extract appear to be primarily mediated by the stimulation of the GAD activity. Indeed, these doses of the extract failed to inhibit the GABA-T activity, in contrast to the highest dose of the extract (200 mg/kg), which induced a significant inhibition of GABA-T activity without increasing GABA concentration. Thus, these findings suggest an interference of the extract with GABA metabolism, likely through GAD activity stimulation (Krespan et al., 1982; Ueda et al., 2001; Sperk et al., 2003). These activities of the extract were comparable to those of sodium valproate, a standard antiepileptic drug, which is known to increase GABA concentration by stimulating the GAD activity or inhibiting the GABA-T activity (Gale, 1992; Treiman, 2001). Furthermore, these data corroborate those of Moto et al. (2018) and Kandeda et al. (2022) in animal models of TLE. According to these authors, administration of the aqueous extract *Lantana camara* or *Cissus quadrangularis* to rodents was followed by an increase in the concentration of GABA in the hippocampus. They thus suggested the role of bioactive molecules in the extract with a potential GABA enhancing effect. Since the decrease in GABA concentration is often associated with neuronal loss, these findings equally suggest a protective effect of the extract against the loss of GABAergic neurons in the hippocampus (Ortiz et al., 2001; Gálvez et al., 2015; Sun et al., 2019). *In vivo* and *in vitro* studies are needed to unravel the exact mechanisms of action of the extract on GABAergic neurotransmission.

Accumulating evidence showed that recurrent or prolonged seizures during TLE are associated with the overproduction of ROS or free radicals (Hsieh et al., 2011; Shin et al., 2011; Puttachary et al., 2015). This phenomenon called excitotoxicity could cause the alteration of neurons and subsequent hyperexcitability of neurons (Cheng et al., 2004; Hsieh et al., 2011; Kandeda et al., 2017a, 2017b). Accordingly, drugs with a preventive effect on oxidative stress could help reduce or inhibit seizures in patients with TLE. In the present study, kainate-induced SE caused significant oxidative stress in the hippocampus of the KA + DW group compared to the DW + DW group. This effect was characterized by an increase in MDA and SOD concentrations and a decrease in GSH and NO concentrations. It is well known that kainate-induced SE increases receptor turn-over, their trafficking to the postsynaptic membrane, and glutamate receptors subunits interactions (Cheng et al., 2004; Hsieh et al., 2011; Shin et al., 2011). These events lead to rapid calcium overload and influx. The activation of signaling pathways by calcium results in a decrease in ATP and an increase in ROS mitochondrial swelling or free radicals (Cheng et al., 2004; Hsieh et al., 2011; Shin et al., 2011). ROS and free radicals interact with all molecules in the cells and cause oxidization of lipid with the increase in the MDA concentration; depletion of GSH, one of the most abundant antioxidants in the central nervous system; and a decrease in total protein because of associated neuronal alterations (Gilberti and Trombetta, 2000; Hsieh et al., 2011; Shin et al., 2011; Kandeda et al., 2021a). Kainate-induced excitotoxicity leads also to an increase in the activity of SOD, an enzyme that catalyzes the dismutation of superoxide anion (O2-) into oxygen (O_2) and peroxide of hydrogen (H_2O_2) (Gilberti and Trombetta, 2000; Cheng et al., 2004). On the other hand, it is established that following kainate-induced excitotoxicity, NO concentration is significantly increased in the hippocampus (Gilberti and Trombetta, 2000; Araujo et al., 2003; Hsieh et al., 2011; Shin et al., 2011). However, the proteolysis of neuronal NO synthase by ROS and free radicals limits the involvement of NO in kainate-induced neurotoxicity in neurons (Araujo et al., 2003). In the present study, the extract at all doses remarkably reduced the concentrations of MDA and SOD suggesting the depletion of free radicals or stimulation of the activity of the antioxidant enzymes (Gilberti and Trombetta, 2000; Araujo et al., 2003; Hsieh et al., 2011; Shin et al., 2011). The extract also increased the concentration of GSH, suggesting free radicals or ROS scavenging activity. Surprisingly, the extract markedly increased the concentration of nitrite (NO). This effect could likely pass through the scavenging of free radicals or inhibition of NO synthetase, an enzyme involved in the synthesis of NO in the neurons (Gilberti

and Trombetta, 2000; Araujo et al., 2003). All these biochemical changes suggest an antioxidant potential of the aqueous extract, likely through free radicals scavenging or antioxidant defense stimulation (Hsieh et al., 2011; Shin et al., 2011; Puttachary et al., 2015). Assuming that the reduction of ROS and free radicals are indicators of the presence of bioactive molecules with antioxidant activities (Allah et al., 2018; Moto et al., 2018), the presence of secondary metabolites (flavonoids and polyphenols) with antioxidant potential might explain these properties of the extract (Marius et al., 2016; Allah et al., 2018). Indeed, the qualitative and quantitative phytochemical analysis of the extract revealed the presence of tannins, coumarins, phenols, triterpenes, and flavonoids, all of which are known to possess antioxidant potential (Audu-Peter et al., 2006; Kolawole et al., 2013; Atawodi et al., 2014; Marius et al., 2016). Due to the documented preventive effect of antioxidants against neuronal alterations or loss, these findings also highlight the neuroprotective effect of the extract (Frantseva et al., 2000; Gupta et al., 2003).

Neuronal alterations in the dentate gyrus are associated with seizures and anxiety (Gröticke et al., 2007). Thus, preventing or reducing this damage has been shown to reduce or prevent seizures and anxiety-like behavior in rodents (Umpierre et al., 2014; Clary et al., 2020).

In the dentate gyrus of the KA + DW group, kainate-induced SE caused a reduced neuronal density, perivascular edema, and granulovascular degeneration compared to the DW + DW group. These alterations were confirmed by a decrease in the number of surviving neurons.

Kainate-induced SE leads to altered behavioral events, which are followed by neurodegeneration in some brain regions, such as the piriform cortex, hippocampus, amygdala complex, and thalamus (Azcoitia et al., 1998; Buckmaster and Jongen-Rêlo, 1999). In the hippocampus, interneurons in the hilus of the dentate gyrus and the CA3 pyramidal cells are the most vulnerable to excitotoxicity (Buckmaster and Jongen-Rêlo, 1999; Maia et al., 2014). Despite the exact molecular mechanisms underlying neuronal loss in these regions being unclear, the activation of astrocytes and microglia, oxidative stress, and proinflammatory cytokines may be involved in SE-induced neuronal loss (Azcoitia et al., 1998; Buckmaster and Jongen-Rêlo, 1999). Histological analysis of dentate gyrus of rats treated with the extract (100 and 200 mg/kg) revealed a reduction in perivascular edema, granulovascular degeneration, and neuronal loss and suggested a neuroprotective effect of the extract (Azcoitia et al., 1998; Buckmaster and Jongen-Rêlo, 1999; Kandeda et al., 2021a,b). These changes were confirmed by the higher number of surviving neurons compared to the KA + DW group. In addition, these effects were similar to those observed with sodium valproate, an antiepileptic drug with neuroprotective properties (Yu et al., 2012; Khamse et al., 2015). The presence of some bioactive molecules in the extract such as seneganolide A and khasenegasin G could account for the neuroprotective effects of the extract. Indeed, these compounds have shown to be neuroprotective *in vitro* against glutamate-induced injury (Olatunji et al., 2021). Also, the main chemical groups in this plant such as alkaloids, tannins, phenols, saponins, sterols, triterpenes, flavonoids could account for the neuroprotective effect of the extract (Atwodi et al., 2014; Marius et al., 2016; Allah et al., 2018). Overall, these results support the above-mentioned antiepileptic- and anxiolytic-like effects of this extract. Furthermore, these findings warrant further investigation on the bioactive compounds present in the extract with potential neuroprotective activity.

5. Conclusion

This study aimed to assess the antiepileptic and anxiolytic-like effects of an aqueous extract of *K. senegalensis* roots, using the kainate model of TLE in rats. The data reveal that the extract prevented spontaneous recurrent seizures and reduced anxiety-like behavior in rats. These effects were greater than those of sodium valproate or phenobarbital, a standard antiepileptic drug. These findings, therefore, suggest antiepileptic- and anxiolytic-like effects. Furthermore, biochemical analyses indicate that these effects are likely mediated in part by GABA modulation and antioxidant activity. These hypotheses were confirmed by the protective activity of the extract against neuronal loss in the dentate gyrus. The findings provide new directions for testing potential antiepileptic, anxiolytic, and neuroprotective properties of the bioactive compounds present in the extract of *K. senegalensis*.

Declarations

Author contribution statement

Antoine Kavaye Kandeda: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stéphanie Lewale: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Etienne Djeuzong: Performed the experiments; Contributed reagents, materials, analysis tools or data.

J. Kouamouo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Théophile Dimo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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