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Ethanolic and aqueous extracts of *Lantana camara* show antiepileptic and anxiolytic effects by inhibiting the ferroptosis pathway in kainate-treated mice

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ABSTRACT

In Cameroon, epilepsy is one of the most common neurological diseases. Available anti-epileptic medication, on the other hand, have been associated with pharmacological toxicity and emotional impairment. The identification of a more efficient replacement is critical. Recent research reveals that ferroptosis contributes to the pathophysiology of epilepsy and related anxiety disorders. *Lantana camara* is a plant with a high neuropharmacological potential, but its mechanisms of action have yet to be understood. The purpose of this study was to determine the effect of ethanolic and aqueous extracts of *Lantana camara* on the kainate model of epilepsy in mice. The focus was on these extracts' capacity to suppress ferroptosis. Mice were injected with kainate (12 mg/kg, i.p.) to induce epilepsy. After *status epilepticus*, animals were left for 19 days, which correspond to an epileptogenic period. After the appearance of spontaneous recurrent seizures, mice were treated with distilled water (10 ml/kg, *p.o.*), levetiracetam (80 mg/kg, *p.o.*), sodium valproate (300 mg/kg, *p.o.*), ethanolic extract of *L. camara* (230, 460, 920 mg/kg, *p.o.*), or an aqueous extract of *L. camara* (460 mg/kg *p.o.*). These treatments lasted for 14 days. During this period, the number and duration of seizures were recorded. The mice were then subjected to elevated zero-maze and open field tests to assess anxiety-like behavior. At the end, mice were sacrificed and hippocampus, amygdala, and striatum were dissected out for biochemical and histological analyses. The extracts alleviated seizure- and anxiety-like behavior in KA-treated mice. Decreased iron levels, reflected by a decrease in ferritin levels and a increase in transferrin levels, were observed in the hippocampus, striatum and amygdala of the extract-treated group compared to the KA-treated group. In addition, increase in GABA and GSH levels, and a decrease in MDA levels were observed in these groups. Hematoxylin-eosin staining revealed less pronounced neuronal degeneration and a more sustained architecture in the brain region of extract-treated mice. These findings indicated that ethanolic and aqueous extracts of *L. camara* effectively attenuate seizures and anxiety disorders. Probable mechanisms of action include GABAergic, iron, GSH, and MDA modulations.

1. Introduction

Epilepsy can be defined as a chronic brain disorder that features recurrent and unprovoked seizures (Alva-Díaz et al., 2021). More than 65 million people worldwide suffer from epilepsy, the most common form being temporal lobe epilepsy (Vezzani et al., 2016; Formentin et al., 2023). This condition is highly prevalent in low- and middle-income nations, with Cameroon being one of the most impacted (Prischich et al., 2008; Njamnshi et al., 2009). This high incidence may be attributed to the lack of access to diagnosis and treatment among the population in this country (Bekele et al., 2024). Over time, several theories have attempted to explain the deleterious mechanism behind epilepsy. Well-accepted theories indicate excitotoxicity, iron overload, oxidative stress, and a decrease in inhibitory drive (Falco-Walter, 2020).

Abbreviations: AE, aqueous extract, ANOVA, analysis of variance; CA, *Cornu Ammonis*; DW, distilled water; DG, dentate gyrus; EE, ethanolic extract; L-GAD, L-glutamate decarboxylase; GABA, γ-aminobutyric acid; GSH, reduced glutathione; KA, kainate; *L. camara*, *Lantana camara*; Lev, Levetiracetam; SEM, standard error of the mean; MDA, malondialdehyde; TLE, Temporal lobe epilepsy; I.p., intraperitoneally; *p.o.*, *per os*; Val, Sodium valproate.

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Furthermore, there is a high prevalence of anxiety disorders in individuals with epilepsy. This is likely due to the anatomical and physiological proximity between the regions of the brain involved in the control of anxiety and those affected by seizures (Vinti et al., 2021).

Epilepsy is caused by genetic and environmental factors. Environmental factors include exposure to neurotoxins such as kainate (KA). The KA model of temporal lobe epilepsy in rodents is well recognized (Lapouble et al., 2006; Mojarad and Roghani, 2014). In addition, the involvement of ferroptosis in seizure-induced cell death in rats and the cognitive comorbidities of temporal lobe epilepsy was reported by Ye et al. (Ye et al., 2019). Since then, ferroptosis's role in the neurodegeneration underlying epilepsy and related neurobehavioral disorders has been discussed in many other studies (Li and Mao, 2019; Mao et al., 2019). Dixon *et al.* reported ferroptosis as a non-apoptotic form of cell death. He discovered that ferroptosis has a special mix of morphological and biochemical features that distinguishes it from autophagy, necrosis and apoptosis (Dixon et al., 2012). Levetiracetam and sodium valproate are two examples of antiepileptic medications that are accessible, although they are linked to drug toxicity and anomalies in the nervous system. The latter has been reported to have anti-ferroptotic activities (Perucca and Gilliam, 2012; Li et al., 2020). Furthermore, these drugs don't address the disease's fundamental causes. Thus, finding a medication that provides a better prognosis is a challenge.

"Inhibition of ferroptosis plays neuroprotective roles in epilepsy and anxiety disorders," a claim made by some scientists, was the starting point for this work (Ye et al., 2019; Xie et al., 2022; Cai and Yang, 2021). Phytomedicine has gained popularity as a dependable source of preparing new drugs to treat various ailments. These plants are readily available, cost-effective, and have milder side effects than chemical drugs. Over the years, *Lantana camara* has produced some interesting results. According to Cameroonian traditional healers, this plant effectively mitigates epilepsy and its associated conditions. Some traditional healers suggest the use of an aqueous decoction, while others suggest maceration of the leaf part of the plant in alcohol for 8 h (personal communication). The plant's aqueous leaf extract showed encouraging antiepileptic properties, according to Kandeda *et al.* (Kandeda et al., 2022). Studies by other authors suggest that the ethanolic extract may potentially contain more secondary metabolites (Ghisalberti, 2000; Tadesse et al., 2017). Furthermore, ethanolic extracts of *L. camara* showed anticonvulsant, anxiolytic, and antidepressant-like properties in neuropharmacological investigations (Kalita et al., 2012; Kazmi et al., 2012; Imran Kazmi et al., 2013; Naz and Bano, 2013; M et al., 2015; Amoah et al., 2023). While current research indicates that ferroptosis plays a role in the etiology of epilepsy, the effects of this herb's extracts on this pathway have yet to be investigated. Thus, the objective of this study was to explore the effects of ethanolic and aqueous extracts of *L. camara* on a kainate model of epilepsy and anxiety disorders. The effects of ethanolic and aqueous extracts on some ferroptosis-related brain markers were highlighted.

2. 1. Materials and methods

2.1. 1.1. Plant collection and extraction

Fresh leaves of *L. camara* were harvested in Yaoundé (Center region, Cameroon). To ensure accurate identification, the plant was compared to a reference specimen deposited at the National Herbarium of Cameroon (HNC) under voucher number 30440/HNC/Cam. This verification was performed by Mr. Ngansop Eric. The plant name was then checked at http://www.theplantlist.org/tpl1.1/record/kew-107934.

The harvested leaves were washed and dried in the shade for 30 days. They were then powdered using a mechanical blender (TT-I777). Two extracts of *L. camara* were used in this study. They were prepared strictly as described by traditional healers. The ethanolic extract was prepared by macerating 20 g of powder in 80 ml of 95 % ethanol for 8 h. The mixture was then filtered using Whatman's paper No 3, and the solvent evaporated at 45°C, yielding 4.43 g of dry extract (22.15 % yield). The aqueous extract was prepared following the method described by in our previous study (Kandeda et al., 2022). The most effective dose (460 mg/kg) from that study was used here. For the current experiment, stock solutions of 230, 460, and 920 mg/kg were prepared for the ethanolic extract. All extracts were administered orally to the mice at a volume of 10 ml/kg body weight.

2.2. 1.2. Drugs, chemicals, and reagents

The study used a variety of chemicals, including kainate, diazepam, valproate, and levetiracetam, all of which were obtained from Sigma Aldrich, Hamburg, Germany. Additionally, trichloroacetic acid and thiobarbituric acid were purchased from Rhone-Poulenc laboratories in Lyon, France, while diazepam was acquired from La Roche laboratories in Switzerland. Other essential reagents, such as Ellman reagent and formalin, were purchased from Sigma Chemical Co., St. Louis, United States.

2.3. 1.3. Animals and ethics

The study utilized male Swiss mice aged 6–8 weeks, weighing 27–33 g. These mice were housed in the Laboratory of Animal Physiology at the University of Yaoundé I in Cameroon under controlled conditions, including a 12-hour light/dark cycle and a temperature range of 24–26 °C. Standard animal diet and tap water were provided *ad libitum*. All animal procedures adhered to ethical guidelines established by the Institutional Ethics Committee of the Cameroon's Ministry of Scientific Research and Technological Innovation (Reg. no. FWA-IRD 0001954, 04/09/2006), which align with the European Union's guidelines on animal care (C.E.E. Council 86/609). Euthanasia was performed following the American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals (2020). The study design, including animal allocation to groups, experimental dosages, outcome measures, and statistical methods, followed the ARRIVE Guidelines 2.0. (https://www.nc3rs.org.uk/arriveguidelines/resources/author-checklists).

2.4. 1.4. Experimental design

2.4.1. 1.4.1. Induction of epilepsy

To induce *status epilepticus*, 82 male mice were randomly divided into two groups as follows:

- a group of 75 mice treated with kainate (12 mg/kg, i.p.);
- a normal control group of 7 mice given 0.9 % saline (10 ml/kg, i.p.).

Two hours after the onset of *status epilepticus,* the mice were treated with diazepam (10 ml/kg, i.p.) for termination of *status epilepticus.* Then, these mice were video-monitored (6 hr/day) for 19 days, until the appearance of spontaneous recurrent seizures (SRS).

2.4.2. 1.4.2. Selection, grouping, and treatment of animals

Mice that developed SRS characterized by tonic-clonic seizures were selected for further studies, then divided into 7 groups of 7 each and treated as follows:

- a kainate group that received 0.9 % saline (10 ml/kg, *p.o.*);
- two positive control groups that either received sodium valproate (300 mg/kg, *p.o.*) or levetiracetam (80 mg/kg, *p.o.*);
- three test groups received the ethanolic extract of *L. camara* at doses of 230, 460, and 917 mg/kg, respectively, *p.o.*;
- another test group received an aqueous extract of *L. camara* at doses of 460 mg/kg, *p.o.*
-a normal control group was added (n = 7), and these animals were treated with 0.9 % saline (10 ml/kg, *p.o.*).

The treatments were administered for 14 days. During this time, the mice were monitored for 6 h a day to record the number and duration of SRS. The severity of seizures was assessed according to the Racine's

scale (Racine, 1972):

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

Twenty-four hours after the end of behavioral seizures analysis, mice were evaluated for anxiety disorders (open-field and elevated zero-maze tests). After these behavioral evaluations, the mice were euthanized and their brains were sampled (Fig. 1).

2.5. Behavioral studies

2.5.1. Elevated zero-maze test

The elevated zero maze is used to assess levels of anxiety, emotionality and reactivity (Tucker and McCabe, 2021). For habituation purposes, animals were placed in the testing room for one hour before the test. The elevated zero-maze used was a wooden apparatus. The apparatus is composed of 2 circular closed arms (50 × 5 × 15 cm) and 2 circular opened arms (50 × 5 × 2 cm). This apparatus was elevated 50 cm from the floor. The mice were placed at the entrance of a closed arm and allowed to explore the maze for 5 min. After each trial, the maze was cleaned with 70° alcohol. The following metrics were tracked with ANY-maze software version 7.2: distance traveled, average speed, number of entries in the closed arm, and percentage of time spent in the opened arm.

2.5.2. Open-field test

The open-field test is commonly used to evaluate the level of locomotor activity, exploration, and emotional reactivity in rodents (Bronikowski et al., 2001). The method used in the present study was that described by Rex et al. (1998). The open arena is made of wood with dimensions of 20 cm × 20 cm × 45 cm (length × width × height). The exploratory surface was subdivided into 17 squares (5 cm × 5 cm) and one central square (5 cm × 5 cm). The mice were placed one after the other in the center of the experimental device. A food lid (5 cm in diameter), containing 5 g of food, was placed at the center of the maze to stimulate exploration. The behavior of each mouse was monitored for 5 min. Distance traveled, number of crossings (number of lines traversed), number of entries, percent time spent in the zones, and the animal's tract plots were all measured in the maze's central and peripheral zones. The latency to first access into the food cover zone and exploratory behavior were also assessed. Any-maze software version 7.2 was used to conduct the analyses.

2.6. Sacrifice and preparation of homogenates

Following the behavioral assessments, animals were immediately euthanized under anesthesia with ketamine (70 mg/kg, i.p.) or diazepam (10 mg/kg, i.p.). Some brains (n = 5) were isolated, washed in 0.9 % NaCl, and blotted for dryness. The hippocampus, amygdala, and striatum were isolated, weighed, and homogenized in Tris-HCl buffer (50 mM, pH 7.4) at a ratio of 20 % (*w/v*). After centrifugation at 3000 rpm at 4 °C for 25 min, the supernatant was collected into tubes and stored at −20 °C for further biochemical evaluations. Other brains (n = 2) were fixed in 10 % formalin for histological analyses.

2.7. Biochemical analyses

The level of GABA in the homogenates was assessed using the Lowe et al. (1988) method. The estimation of reduced glutathione (GSH) and malondialdehyde (MDA) levels was performed as described by Ellman (Ellman, 1959) and Wilbur *et al.* (Wilbur et al., 1949), respectively. The iron, ferritin, and transferrin levels were assessed using colorimetric kits purchased from LABKIT®.

2.8. Histological analyses

This study followed a standardized histological protocol adapted from Suvarna *et al.* (Survana et al., 2019) to analyze the microarchitecture of the CA1 and dentate gyrus of the hippocampus, striatum (Caudate Putamen), and amygdala (basolateral nucleus) in the brain of mice. This protocol involved a series of steps: tissue fixation, sectioning, dehydration, embedding, trimming, staining using hematoxylin and eosin, mounting, and finally, observation under a microscope. The stained and mounted slides were examined at 100× magnification using a Scientico STM-50 optical microscope equipped with a digital camera for image capture. Image analysis was then performed using Image J software version 1.54i.

2.9. Statistics

GraphPad Prism version 8.01 and Microsoft Office Excel 2019 version 15.0.4420.1017 were used for statistical analysis of the data obtained, as well as graph construction. Results were reported as mean ± SEM or percentages. The various values were compared using a one- or two-way analysis of variance (ANOVA). When differences emerged, the Tukey multiple comparison post-hoc tests were utilized. The difference was statistically significant at $p < 0.05$.

3. Results

3.1. Effects of the extracts of Lantana camara on the number and duration of seizures

3.1.1. Effects on the mean number of seizures

Epilepsy is characterized by spontaneous and recurrent seizures. No seizures were observed in the control group. The administration of kainate provoked stages 1, 2, 3, and 4 seizures in all mice. The ethanolic extract (920 mg/kg) of the plant led to a significant decrease ($p < 0.001$) in the mean number of stages 1 by 75.75 %, stage 2 by 75.86 %, and stage 3 by 87.39 % seizures, relative to negative control. In addition, this extract at all doses, prevented the appearance of stage 4 seizures. On the other hand, the administration of the aqueous extract led to a significant decrease in the mean number of stages 1 by 72.31 %, stage 2 by 75.86, stage 3 seizures by 87.39 %, and prevented the appearance of stage 4. The administration of levetiracetam led to a significant ($p < 0.001$) decrease in the mean number of stages 1 by 69.23 %, stage 2 by 77.59 %, stage 3 seizures by 86.41 %, and prevented the appearance of stage 4 seizures. The administration of sodium valproate led to a significant ($p <$

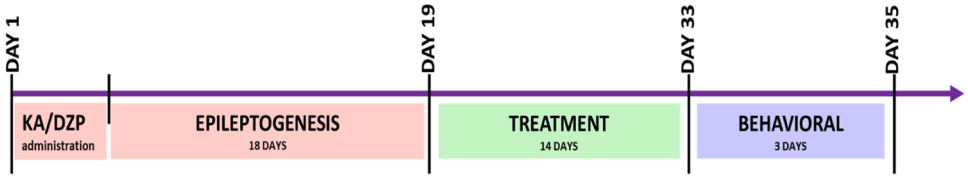

Fig. 1. Experimental protocol. KA, kainate (12 mg/kg); DZP, diazepam (10 ml/kg).

0.001) decrease in the mean number of stages 1 by 59.23 %, stage 2 by 65.52 %, stage 3 seizures by 74.76 %, and prevented the appearance of stage 4 seizures (Fig. 2).

3.1.2. *Effects on the mean duration of seizures*

Fig. 3 shows the mean duration of seizures *per* day of treatment. The administration of kainate significantly increased ($p < 0.001$) the mean duration of stages 1, 2, 3, and 4 seizures in mice, compared to all groups. The ethanolic extract (460 mg/kg) led to a significant decrease in the duration of stages 1 by 76.93 %, stage 2 by 81.25 %, and stage 3 by 85.42 % seizures, relative to the kainate-treated group. On the other hand, the administration of the aqueous extract led to a significant decrease in the daily duration of stages 1 by 72.30 %, stage 2 by 81.73 %, and stage 3 by 86.44 %. The administration of levetiracetam led to a significant decrease ($p < 0.001$) in the duration of stages 1 by 69.22 %, stage 2 by 81.88 %, and stage 3 seizures by 96.13 %. The administration of sodium valproate led to a significant decrease in the duration of stages 1 by 59.24 %, stage 2 by 74.12 %, and stage 3 seizures by 74.79 % (Fig. 3) as compared to the kainate-treated group.

3.2. *Effects of the extracts of Lantana camara on anxiety-like behavior and exploratory activities in the elevated zero-maze*

3.2.1. *Effects of the extracts of Lantana camara on anxiety-like behavior in the elevated zero-maze*

Anxiety disorders are associated with epilepsy. The distance traveled in the maze by mice treated with kainate was significantly reduced by 46.43 % ($p < 0.01$) than that of the normal control. The ethanolic extract at the dose of 460 mg/kg showed an increase in this distance by 112.26 % ($p < 0.001$), while the aqueous extract showed an increase of 98.87 % ($p < 0.001$) when compared to the kainate-treated group. Sodium valproate caused an increase in this metric by 75.47 % ($p < 0.01$) relative to the kainate-treated group (Table 1).

The average speed in the maze of mice treated with kainate was significantly reduced by 50.75 % ($p < 0.01$) compared to that of the normal control. The ethanolic extract at the dose of 460 mg/kg caused an increase in this parameter by 96.97 % ($p < 0.001$), while the aqueous extract caused an increase by 30.30 % ($p < 0.05$) when compared to the kainate-treated group. Sodium valproate caused an increase in this number by 81.82 % ($p < 0.01$) relative to the kainate-treated group (Table 1).

The number of entries in the closed arm and percentage time spent in the opened arm by mice in the kainate-treated group were significantly reduced by 77.30 % ($p < 0.001$) and 5.56 % ($p < 0.001$), respectively, when compared to the normal control. The ethanolic extract at a dose of 460 mg/kg led to an increase in the number of entries in the closed arm by 143.75 % ($p < 0.05$) and the percentage time spent in the opened arm by 69.94 % ($p < 0.01$). Meanwhile, the aqueous extract led to an increment in these numbers by 50.00 % ($p < 0.05$) and 33.92 % ($p < 0.05$), respectively, when compared to the kainate-treated group (Table 1). Sodium valproate caused an increase in this number to 287.50 % ($p < 0.001$) and 110.08 % ($p < 0.001$) relative to the kainate-

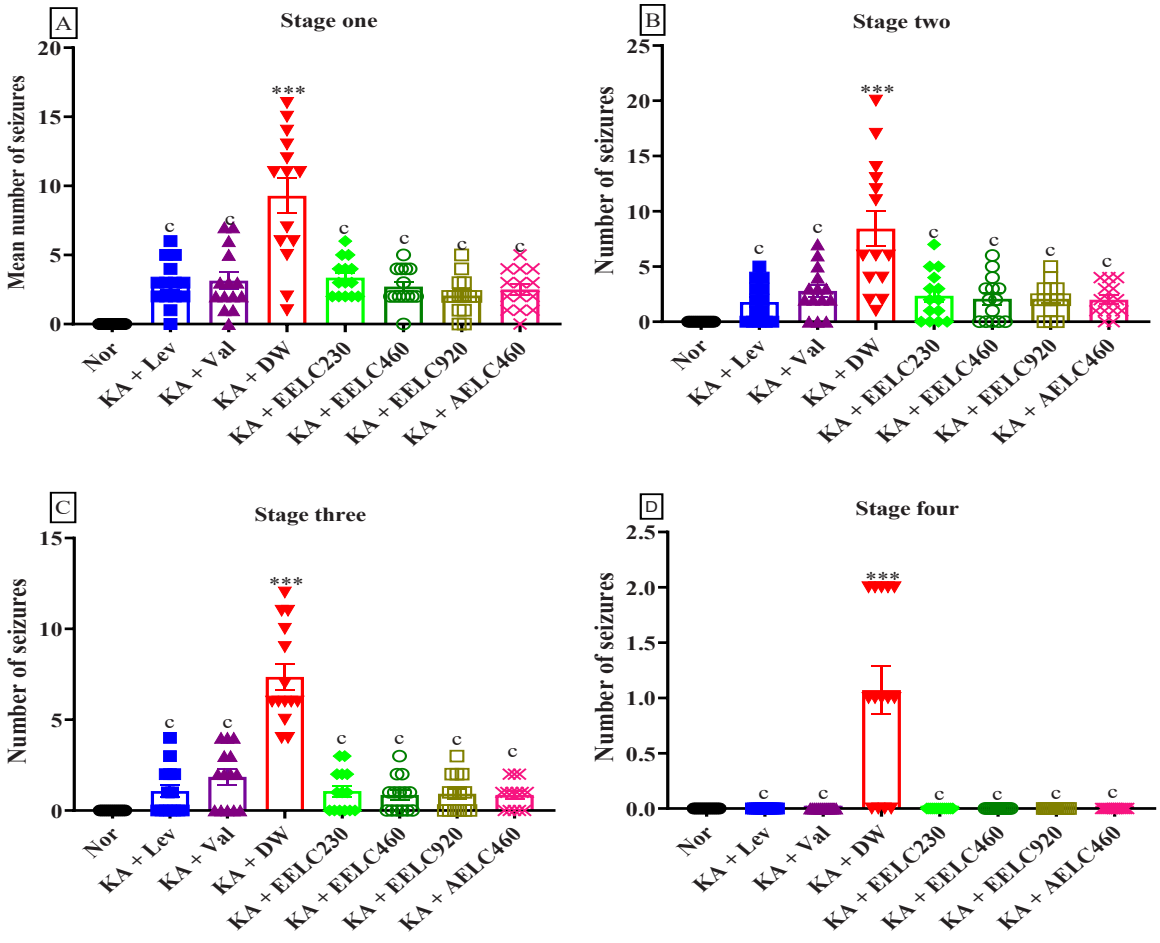

Fig. 2. Effects of the extracts of *Lantana camara* on the average number of seizures *per* day. A: stage one, B: stage two, C: stage three, D: stage four. Results were expressed as mean ± SEM. N = 14. Data were analyzed by one-way ANOVA, followed by Tukey *post hoc* test. ***$p < 0.001$ vs control group treated only with saline 0.9 %; c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

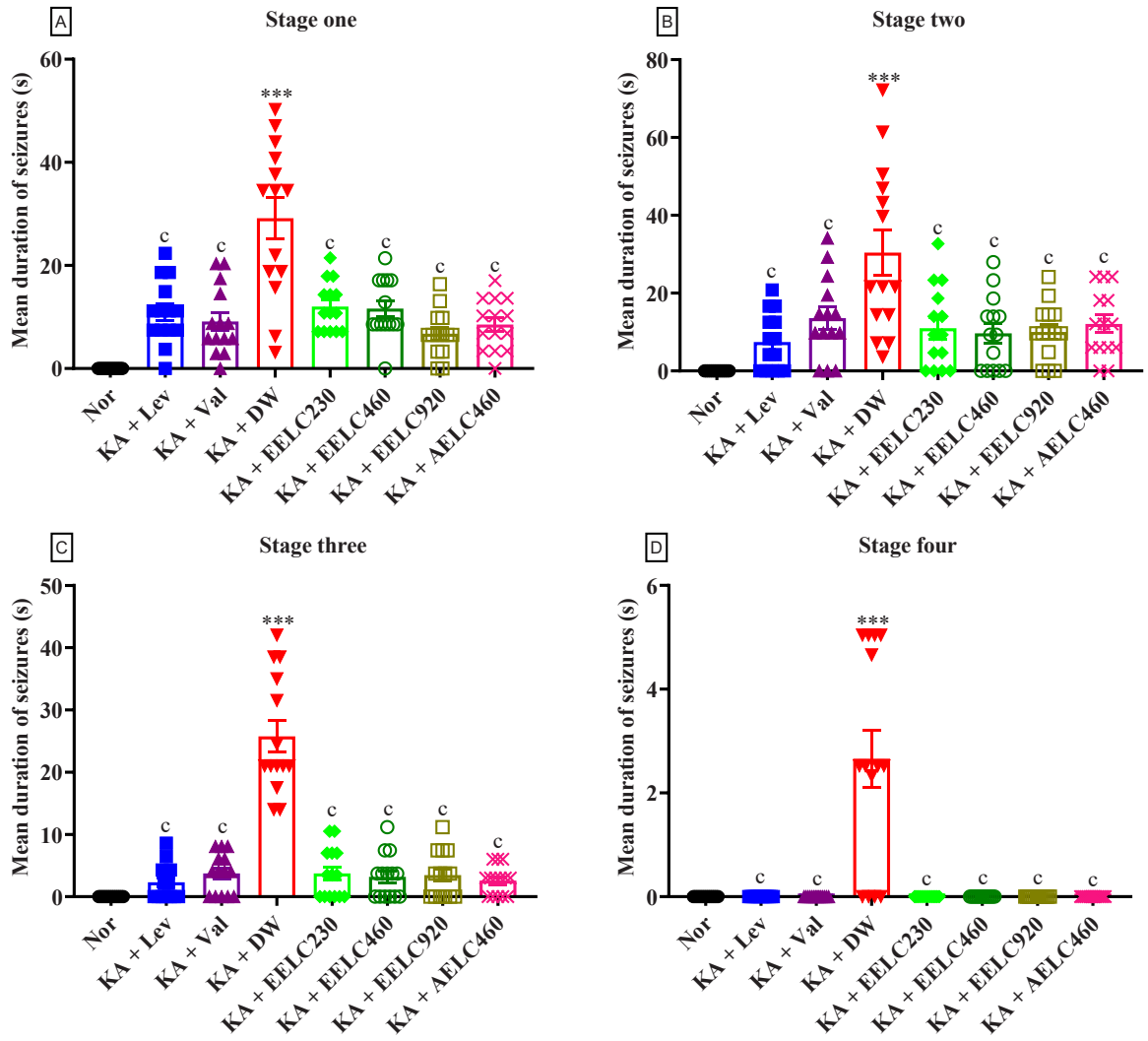

Fig. 3. Effects of the extracts of *Lantana camara* on the mean duration of seizures *per* day. A: stage one, B: stage two, C: stage three, D: stage four seizures. Results were expressed as mean ± SEM. N = 14. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.01$, vs control group, treated only with saline 0.9 %; c$p < 0.001$ vs disease kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg). EE, ethanolic extract; AE, aqueous extract; LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; DW, distilled water (10 ml/kg); Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

treated group (Table 1).

3.2.2. Effects on the exploratory activity of mice in the elevated zero-maze

The elevated zero maze (EZM) was used to examine mice's exploratory behavior. Fig. 4 shows red and blue plots representing mouse movement in the maze's closed and open arms, respectively. Normal control mice demonstrated balanced exploration, spending similar time in both arms (Fig. 4A). In contrast, kainate-treated mice showed a strong bias toward the closed arm, as demonstrated by a more pronounced red plot and a decreased blue plot compared to the control group (Fig. 4D). Notably, the exploratory behavior of mice treated with the extracts (Fig. 4E, F, G, and H), levetiracetam (Fig. 4B), and valproate (Fig. 4C) was similar to that of the normal controls.

3.3. Effects of the extracts of Lantana camara on anxiety-like behavior in the open field

3.3.1. Effects on the distance traveled and exploratory activity of mice

In its upper panel, Fig. 5 shows the distances traveled in the peripheral and central zones of the OF. These numbers were elevated in the normal control group by 48.64 % ($p < 0.001$) and 77.55 % ($p < 0.001$), respectively, compared to the kainate-treated group. This is reflected by the poor exploration of the zones by kainate-treated mice relative to that of the normal control (Lower panel, Fig. 5A, D). The ethanolic extract of the plant at the dose of 460 mg/kg caused an increase in these parameters, respectively, by 179.66 % ($p < 0.001$) and 377.25 % ($p < 0.001$). Meanwhile, the aqueous extract showed an increase of 50.51 % ($p < 0.01$) and 132.28 % ($p < 0.001$) in these respective numbers, when compared to the kainate-treated group. Mice treated with these extracts showed the greatest exploratory activities, both in the peripheral and central zones (Lower panel, Fig. 5E, F, G, and H). Sodium valproate caused an increase in these numbers by 30.85 % ($p < 0.05$) and 205.82 % ($p < 0.001$), respectively, compared to the kainate-treated group.

3.3.2. Effects in the peripheral zone of the open field

Table 2 displays the behavior of mice treated with the extracts in the peripheral zone of the open field. The number of crossings was elevated in the normal control group by 121.48 % ($p < 0.001$), compared to the kainate-treated group. The ethanolic extract of the plant at the dose of 460 mg/kg caused an increase in these parameters by 195.56 % ($p < 0.001$). Meanwhile, the aqueous extract showed an increase of 67.41 %

Table 1
Effects of the extracts of *Lantana camara* on anxiety-like behavior in the elevated zero-maze.

Treatments	Distance traveled (m)	Average speed ($\times 10^{-3}$ m/s)	Number of entries in closed-arm	Percentage time spent in opened arm (%)
Nor	1.97 ± 0.31	6.70 ± 0.70	35.25 ± 2.50	35.13 ± 2.11
KA + Lev	1.93 ± 0.11^{b}	6.50 ± 0.31^{b}	18.00 ± 1.08^{a}	33.06± 2.16^{a}
KA + Val	1.86 ± 0.22^{b}	6.00 ± 0.72^{b}	31.00 ± 4.42^{c}	62.12 ± 4.34^{c}
KA + DW	1.06 ± 0.34**	3.30 ± 1.10**	08.00 ± 2.55***	29.57 ± 3.22*
KA + EELC230	1.90 ± 0.36^{b}	6.50 ± 1.22^{b}	14.25 ± 3.35^{a}	48.63 ± 2.67^{b}
KA + EELC460	2.25 ± 0.49^{c}	7.20 ± 1.70^{b}	19.50 ± 3.48^{a}	50. 25 ± 4.02^{b}
KA + EELC920	2.05 ± 0.24^{c}	7.00 ± 0.74^{b}	15.00 ± 2.20^{a}	44.14 ± 3.22^{b}
KA + AELC460	1.26 ± 0.23^{a}	4.30 ± 0.73^{a}	12.00 ± 2.80^{a}	39.06 ± 2.33^{a}

Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs normal control group treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

($p < 0.05$) when compared to the kainate-treated group. Sodium valproate led an increase in these numbers by 50.64 % ($p < 0.01$), respectively, compared to the kainate-treated group.

The number of entries in the peripheral zone and percentage time spent in this zone were significantly reduced by 58.18 % ($p < 0.001$) and 44.22 % ($p < 0.001$), respectively, in the kainate-treated group as compared to the normal control. The ethanolic extract of the plant at the dose of 920 mg/kg led to an increase in the number of entries by 261.11 % ($p < 0.05$) and the percentage time spent in this zone by 29.56 % ($p < 0.01$). Meanwhile, the aqueous extract led to an increment in these numbers by 150.00 % ($p < 0.001$) and 40.88 % ($p < 0.01$), respectively, when compared to the kainate-treated group (Table 2). Sodium valproate caused an increase ($p < 0.001$) in these numbers by 205.56 % and 42.26 %, relative to the kainate-treated group (Table 2).

3.3.3. *Effects in the central zone of the open field*

The number of entries in the central zone of the OF decreased significantly ($p < 0.001$) by 88.39 % in kainate-treated animals relative to the normal control (Table 3). The ethanolic extract of the plant at the dose of 460 mg/kg caused an increase in the number of entries by 377.25 % ($p < 0.001$). Meanwhile, the aqueous extract led to an increment in these numbers by 132.28 % ($p < 0.05$), when compared to the kainate-treated group (Table 3). Sodium valproate led an increase in this number by 175.00 ($p < 0.001$) relative to the kainate-treated group (Table 3).

A significant decrement ($p < 0.001$) in the latency to the first entry, percentage time spent, and latency to the first entry to the food lid zone ($p < 0.001$) by 204.79 %, 14.08 %, 204.79 %, respectively, was observed in the animals of the kainate-treated group after comparison with those of the normal control group. The ethanolic extract of the plant at the dose of 920 mg/kg led to an increase in the latency to first entry by 66.44 % ($p < 0.001$), percentage time spent in this zone by 27.33 % ($p < 0.01$), and latency to the first entry to the food lid zone 66.44 % ($p < 0.001$) as compared to the animals in the negative control group. Meanwhile, the aqueous extract led to an increment in these numbers to 71.64 % ($p < 0.001$), 37.80 % ($p < 0.01$), and 71.64 % ($p < 0.001$), respectively, when compared to the kainate-treated group (Table 3). Sodium valproate caused a significant increase ($p < 0.001$) in these numbers by 69.67 %, 39.07 %, and 69.67 %, respectively, when compared to the kainate-treated group (Table 3).

3.4. *Effect of the extracts of L. camara extract on GABA levels in the hippocampus, striatum, and amygdala*

GABA is reported to provide an equivalent inhibitory drive to counteract neuronal hyperexcitation and prevent epilepsy and anxiety

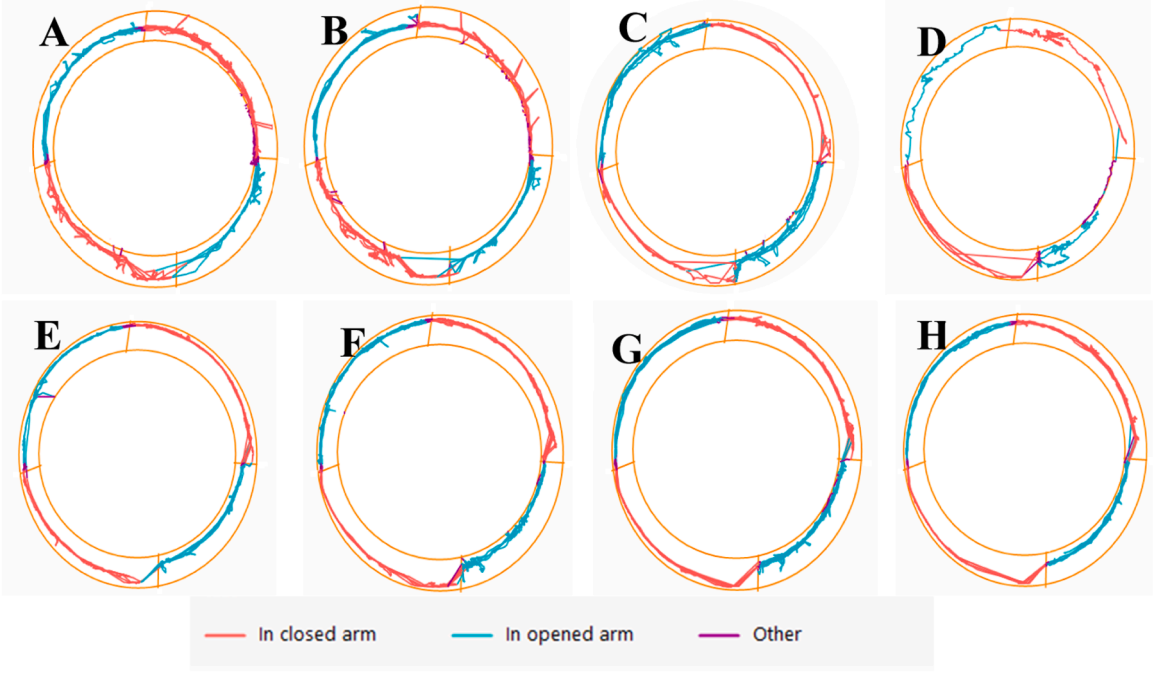

Fig. 4. Exploratory tract plot of mice in the closed arms (red) and opened arms (blue) of the elevated zero maze. Normal control (A), Levetiracetam-treated (B), Valproate-treated (C), Kainate-treated (D), ethanolic extract-treated (E, F, G), and aqueous extract-treated (H) groups. The red plot represents the movement of mice in the closed arm, while the blue plot shows activity in the opened arm.

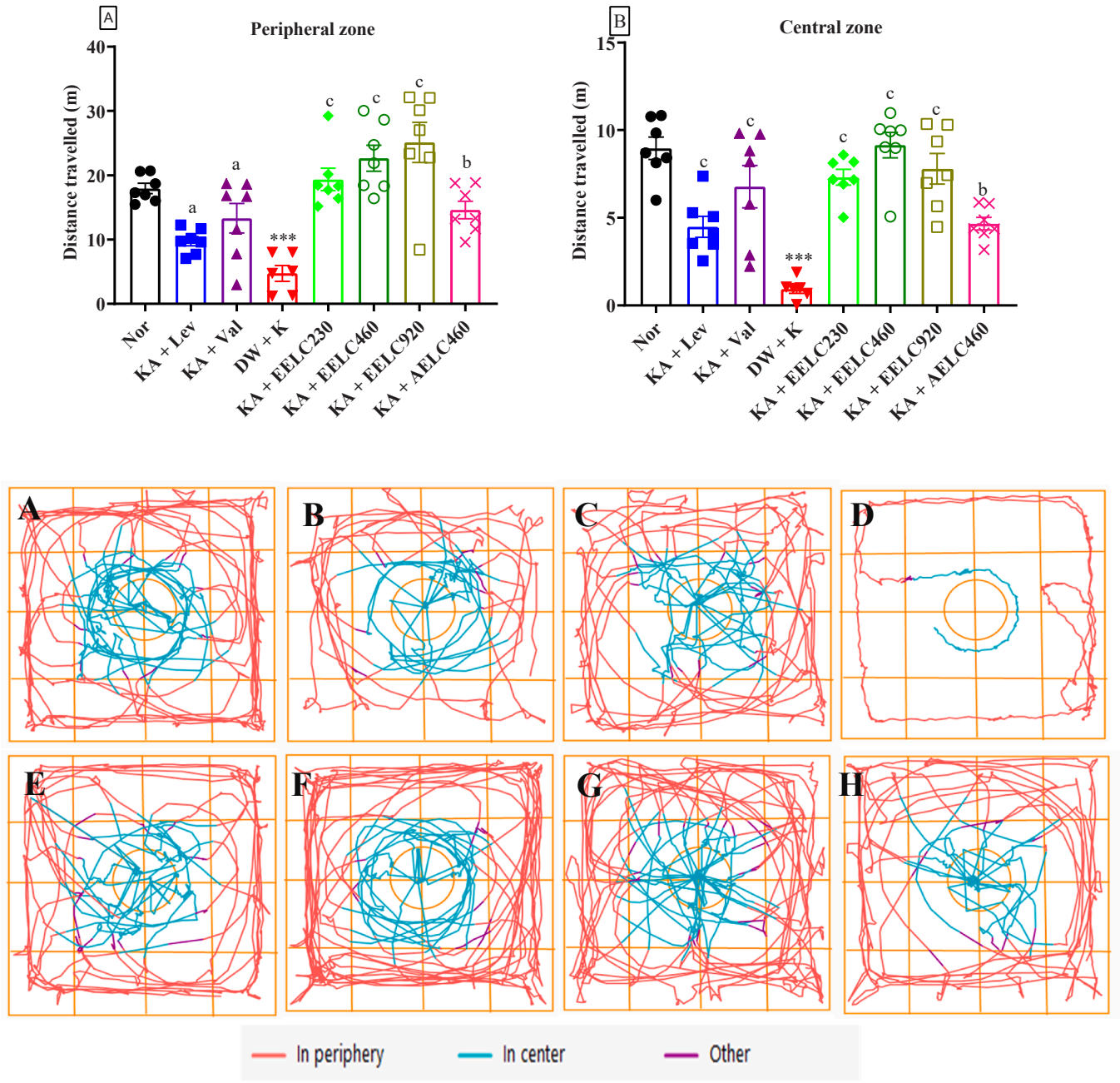

Fig. 5. *Upper panel* – Effects of the extracts of *L. camara* extract on the distance traveled in the peripheral (A) and central (B) zones of the open field. Results were expressed as mean, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.01$, vs control group, treated only with saline 0.9 %; b$p <$ 0.01, c$p < 0.001$ vs disease kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg). EE, ethanolic extract; AE, aqueous extract; LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; DW, distilled water (10 ml/kg); Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg). Red plots indicate a tract plot in the peripheral zone; blue plots indicate a tract of mice in the central zone of the maze.*Lower panel* – Exploratory tract plot of mice in the peripheral (red) and central (blue) zones of the open field. Normal control (A), Levetiracetam-treated (B), Valproate-treated (C), Kainate-treated (D), ethanolic extract-treated (E, F, G), and aqueous extract-treated (H) groups.

disorders. The level of GABA was decreased ($p < 0.001$) in the hippocampus by 63.91 %, amygdala by 46.96 %, and striatum by 93.14 % of animals treated with kainate as compared to normal control animals. This amount was found to increase ($p < 0.001$) in the hippocampus by 177.50 % amygdala by 82.29 %, and striatum by 77.51 % of animals treated with the ethanolic extract (460 mg/kg). Similar observations were made in these zones of the brains of animals treated with the aqueous extract of the plant i.e. hippocampus by 108.53 % ($p < 0.001$), amygdala by 53.81 % ($p < 0.05$), and striatum by 53.68 % ($p < 0.001$). Levetiracetam led to an increase in the level of this neurotransmitter in the hippocampus by72.97 % ($p < 0.01$), amygdala by 32.19 % ($p <$ 0.001), and striatum by 123.63 % ($p < 0.001$), relative to the kainate-treated group. Sodium valproate led to an increase ($p < 0.001$) in the level of this neurotransmitter in the hippocampus by 165.35 %, amygdala by 98.72 %, and striatum by 58.74 % relative to the kainate-treated group (Fig. 6).

3.5. Effects of the extracts of L. camara extract on MDA and GSH levels in the hippocampus, striatum, and amygdala

3.5.1. Effects on GSH levels in the hippocampus, amygdala, and striatum

Reduced glutathione (GSH) can be used to store iron, preventing

Table 2
Effects of the extracts of *Lantana camara* on some parameters in the peripheral zone of the open field.

Treatments	Number of crossings	Number of entries	Percentage of time spent (%)
Nor	74.75 ± 5.72	19.25 ± 1.11	33.31 ± 1.30
KA + Lev	45.75 ± 7.56^{b}	10.00 ± 2.35^{b}	22.51 ± 1.45^{b}
KA + Val	50.75 ± 8.02^{b}	13.75 ± 4.53^{c}	27.74 ± 2.44^{b}
KA + DW	33.75 ± 7.49***	04.50 ± 2.18***	48.04 ± 3.56***
KA + EELC230	85.75 ± 13.62^{c}	16.75 ± 0.85^{c}	43.16 ± 3.44^{c}
KA + EELC460	99.75 ± 11.56^{c}	16.25 ± 1.80^{c}	33.20 ± 3.20^{c}
KA + EELC920	97.25 ± 10.15^{c}	19.75 ± 2.69^{c}	33.84 ± 4.55^{c}
KA + AELC460	56.50 ± 7.96^{c}	11.25 ± 0.48^{c}	28.40 ± 2.22^{b}

Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs normal control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

Table 3
Effects of the extracts of *Lantana camara* in the central zone of the open field.

Treatments	Number of entries	Latency to the first entry (s)	Percentage of time spent (%)	Latency to entry in the food lid zone (s)
Nor	15.50 ± 1.55	18.80 ±10.37	66.69 ± 1.30	18.80 ± 8.78
KA + Lev	8.75 ± 2.02^{b}	26.45 ±6.58^{c}	77.49 ± 1.45^{b}	26.45 ± 4.30^{c}
KA + Val	11.00 ± 3.81^{c}	17.38±2.54^{c}	72.26 ± 2.44^{b}	17.38 ± 3.04^{c}
KA + DW	4.00 ± 1.38***	57.30 ± 7.97***	51.96 ± 3.56***	57.30 ± 22.88***
KA + EELC230	12.75 ± 1.11^{c}	32.77 ±9.37^{b}	57.84 ± 3.44^{c}	32.77 ± 10.22^{c}
KA + EELC460	12.75 ± 1.70^{c}	20.17 ±5.59^{c}	66.16 ± 3.20^{c}	25.17 ± 9.03^{c}
KA + EELC920	16.00 ± 2.48^{c}	19.23 ± 6.67^{c}	66.16 ± 4.55^{c}	19.23 ± 6.80^{c}
KA + AELC460	8.50 ± 0.65^{b}	16.25 ± 5.84^{c}	71.60 ± 2.22^{b}	16.25 ± 5.49^{c}

Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

ferroptosis. Fig. 7 shows a depletion of GSH in the hippocampus by 49.16 % ($p < 0.001$), the amygdala by 83.00 % ($p < 0.001$), and the striatum by 35.91 % ($p < 0.01$), relative to the normal control group. The ethanolic extract of the plant, at a dose of 460 mg/kg, led to an increase in the level of GSH in the hippocampus by 37.95 % ($p < 0.001$), amygdala by 23.90 % ($p < 0.001$), and striatum by 32.16 % ($p < 0.001$). Similar observations were made in the aqueous extract-treated group i.e. an increase in this amount in the hippocampus (58.24 %; $p < 0.001$), amygdala (19.44 %; $p < 0.05$), and striatum (32.00 %; $p < 0.01$) when compared to the kainate-treated group. Levetiracetam led to an increase in the level of GSH in the hippocampus (61.98 %; $p < 0.001$), amygdala (8.8 %; $p < 0.05$), and striatum (52.59 %; $p < 0.001$). Also, Sodium valproate led to an increase in the level of GSH in the hippocampus (75.21 %; $p < 0.001$), amygdala (47.20 %; $p < 0.01$), and striatum (54.00 %; $p < 0.001$), relatively to the kainate-treated group (Fig. 7).

3.5.2. Effects on MDA levels in the hippocampus, amygdala, and striatum

Malondialdehyde (MDA) is a product of ferroptosis-mediated lipid peroxidation. Data from Fig. 8 reveals an increment in the level of MDA in the hippocampus by 151.87 % ($p < 0.001$), amygdala by 83.00 % ($p < 0.001$), and striatum by 93.21 % ($p < 0.01$) of kainate-treated mice, relatively to the normal control group. The ethanolic extract of the plant, at a dose of 460 mg/kg, led to a decrease in the level of MDA in the hippocampus by 50.27 % ($p < 0.001$), amygdala by 50.88 % ($p < 0.001$), and striatum by 47.89 % ($p < 0.001$). Similar observations were made in the aqueous extract-treated group i.e. an increase in this amount in the hippocampus (44.00 %; $p < 0.01$), amygdala (53.46 %; $p < 0.05$), and striatum (46.63 %; $p < 0.001$) when compared to the kainate-treated group. Levetiracetam led to a decrease in the level of MDA in the hippocampus (45.89 %; $p < 0.001$), amygdala (51.02 %; $p < 0.001$), and striatum (48.03 %; $p < 0.001$). Also, sodium valproate led to a decrease in the level of MDA in the hippocampus (48.04 %; $p < 0.001$), amygdala (53.05 %; $p < 0.01$), and striatum (47.44 %; $p < 0.001$), relatively to the kainate-treated group.

3.6. Effect of the extracts of L. camara on iron, ferritin, and transferrin levels in the hippocampus, amygdala, and striatum

3.6.1. Effect on iron levels in the hippocampus, amygdala, and striatum

Accumulation of iron induces ferroptosis and promotes the formation of reactive oxygen species, which is responsible for epilepsy progression. The iron level was found to be increased in the hippocampus by 202.48 % ($p < 0.001$), the amygdala by 176.95 % ($p < 0.001$), and the striatum by 141.74 % ($p < 0.001$) of kainate-treated mice, compared to the normal control. The ethanolic extract of the plant at the dose of 460 mg/kg led to a decrease in the levels of iron in the hippocampus by 55.97 % ($p < 0.001$), amygdala by 57.68 ($p < 0.001$), and striatum by 56.15 % ($p < 0.001$), relative to the kainate group. Similarly, the aqueous extract of the plant led to a decrease in the levels of iron in the hippocampus by 42.16 % ($p < 0.001$), the amygdala by 56.70 ($p < 0.001$), and the striatum by 50.68 % ($p < 0.001$), relative to the kainate group. Levetiracetam led to a decrease in the level of this marker in the hippocampus by 54.10 % ($p < 0.001$), amygdala by 57.68 % ($p < 0.001$), and striatum by 55.90 % ($p < 0.001$), relative to the kainate-treated group. On the other hand, sodium valproate led to a decrease in the level of this marker in the hippocampus by 46.64 % ($p < 0.001$), the amygdala by 56.22 % ($p < 0.001$), and the striatum by 50.99 % ($p < 0.001$), relative to the kainate-treated group (Fig. 9).

3.6.2. Effects on ferritin level in the hippocampus, amygdala, and striatum

Ferritin is an iron storage protein and an indicator of iron overload. Data from Fig. 10 reveals an increase in the level of ferritin in the hippocampus by 110.07 % ($p < 0.001$), amygdala by 414.86 % ($p < 0.001$), and striatum by 300 % ($p < 0.001$) of kainate treated-mice, compared to the normal control. The ethanolic extract of the plant at the dose of 230 mg/kg led to a decrease in the levels of this indicator in the hippocampus by 41.07 % ($p < 0.001$), amygdala by 76.32 % ($p < 0.001$), and striatum by 38.89 % ($p < 0.001$), relative to the kainate group. Also, in comparison to the kainate group, the aqueous extract of the plant led to a decrease in the levels of iron in the hippocampus by 37.5 % ($p < 0.001$), amygdala by 44.74 % ($p < 0.001$), and striatum by 36.11 % ($p < 0.001$). Levetiracetam decreased the level of this marker in the hippocampus by 37.50 % ($p < 0.001$), amygdala by 68.42 % ($p < 0.001$), and striatum by 72.22 % ($p < 0.001$), relative to the kainate-treated group. Sodium valproate decreased in the level of this marker in the hippocampus by 48.21 % ($p < 0.001$), amygdala by 44.74 % ($p < 0.001$), and striatum by 52.78 % ($p < 0.001$), relative to the kainate-treated group (Fig. 10).

3.6.3. Effects on transferrin levels in the hippocampus, amygdala, and striatum

Transferrin is an iron transporter as well as a neuroprotective agent.

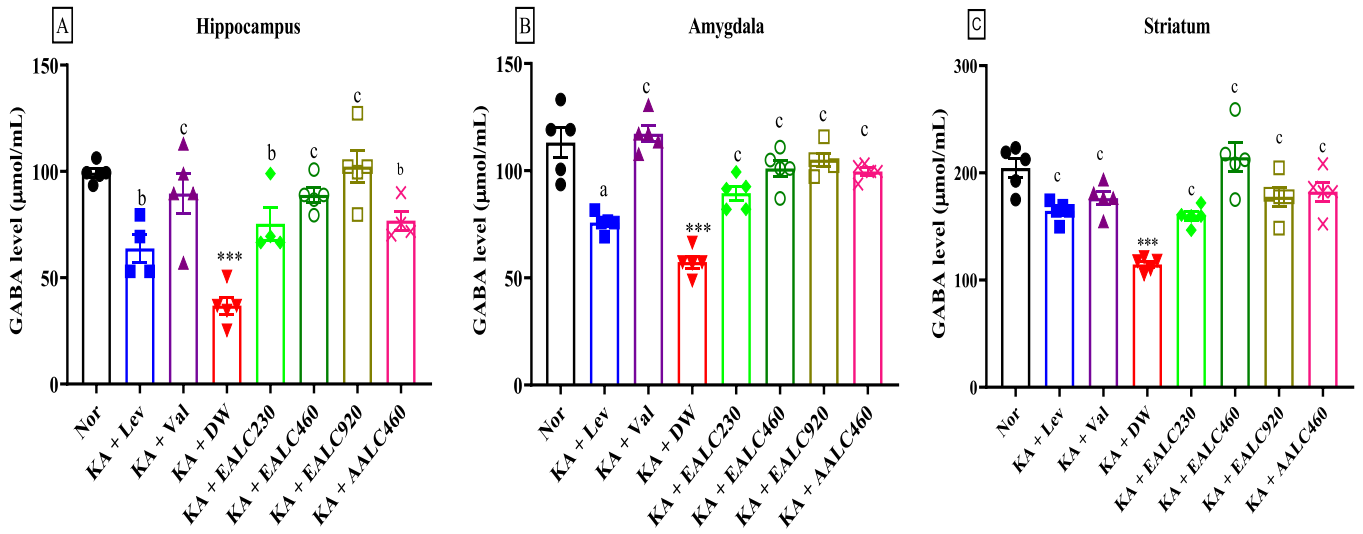

Fig. 6. Effects of the extracts of *L. camara* extract on GABA level in the hippocampus(A), amygdala (B), and striatum (C) Data are mean ± SEM, N = 5 per group. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$ vs control group treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); Nor, control animal treated with distilled water(10 mg/kg); EE, ethanolic extract; AE, aqueous extract; LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg;); Val, sodium valproate (300 mg/kg).

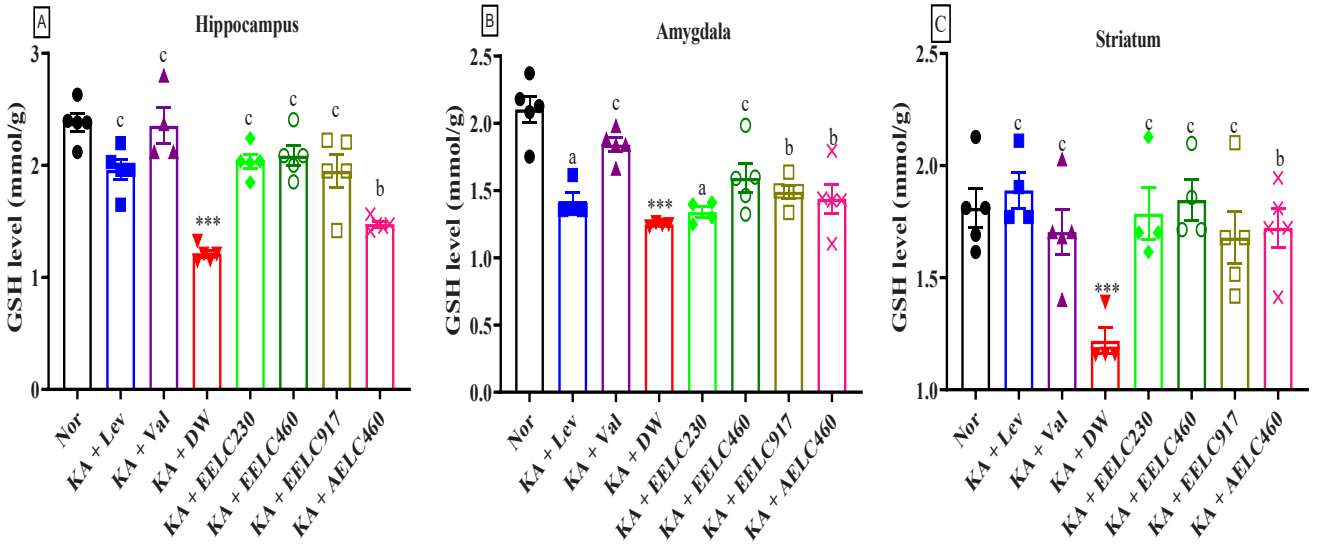

Fig. 7. Effect of the extracts of *L. camara* extract on GSH levels in the hippocampus (A), amygdala (B), and striatum (C). Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

Data from Fig. 11 reveals a decrease in the level of transferrin in the hippocampus by 44.44 % ($p < 0.001$), amygdala by 33.33 % ($p < 0.001$), and striatum by 33.33 % ($p < 0.001$) of kainate-treated mice, compared to the control. The ethanolic extract of the plant at the dose of 230 mg/kg led to an increase in the levels of this protein in the hippocampus by 40.00 % ($p < 0.001$), amygdala by 64.29 % ($p < 0.001$), and striatum by 50.00 % ($p < 0.001$), relative to the kainate group. The aqueous extract of the plant led to an increase in the levels of this protein in the hippocampus by 40.00 % ($p < 0.001$), amygdala by 57.14 % ($p < 0.001$), and striatum by 31.25 % ($p < 0.001$), relative to the kainate group. Levetiracetam led to a decrease in the level of this marker in the hippocampus by 60.0 % ($p < 0.001$), amygdala by 57.14 % ($p < 0.001$), and striatum by 56.25 % ($p < 0.001$), relative to the kainate-treated group. On the other hand, sodium valproate led to a decrease in the level of this marker in the hippocampus by 72.00 % ($p < 0.001$), the amygdala by 57.14 % ($p < 0.001$), and the striatum by 62.50 % ($p < 0.001$), relative to the kainate-treated group (Fig. 11).

3.7. Effects of the extracts of Lantana camara on the histology of the hippocampus, amygdala, and striatum

3.7.1. Effects of the extracts of Lantana camara on the microarchitecture and percentage of degenerating neurons in the Cornu ammonis 1 and dentate gyrus of the hippocampus

Fig. 12 (*upper panel*) shows the hematoxylin-eosin stain of the CA1 and dentate gyrus of the hippocampus. The micro-architecture of the hippocampus of the normal control group showed healthy neurons in the CA1 and DG (Fig. 12 A1 and A2, respectively). Compared to the

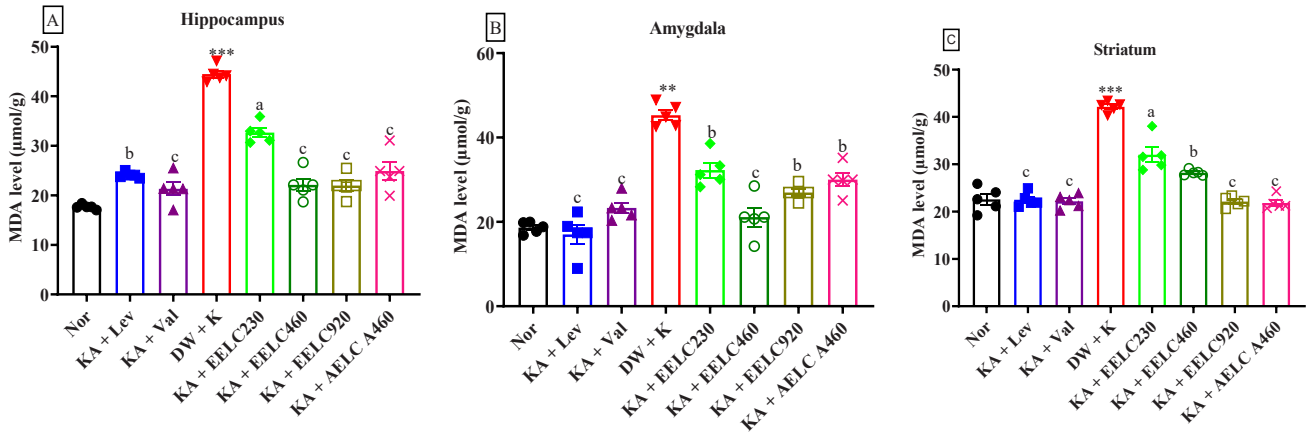

Fig. 8. Effect of the extracts of *L. camara* extract on MDA levels in the hippocampus (A), amygdala (B), and striatum (C). Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

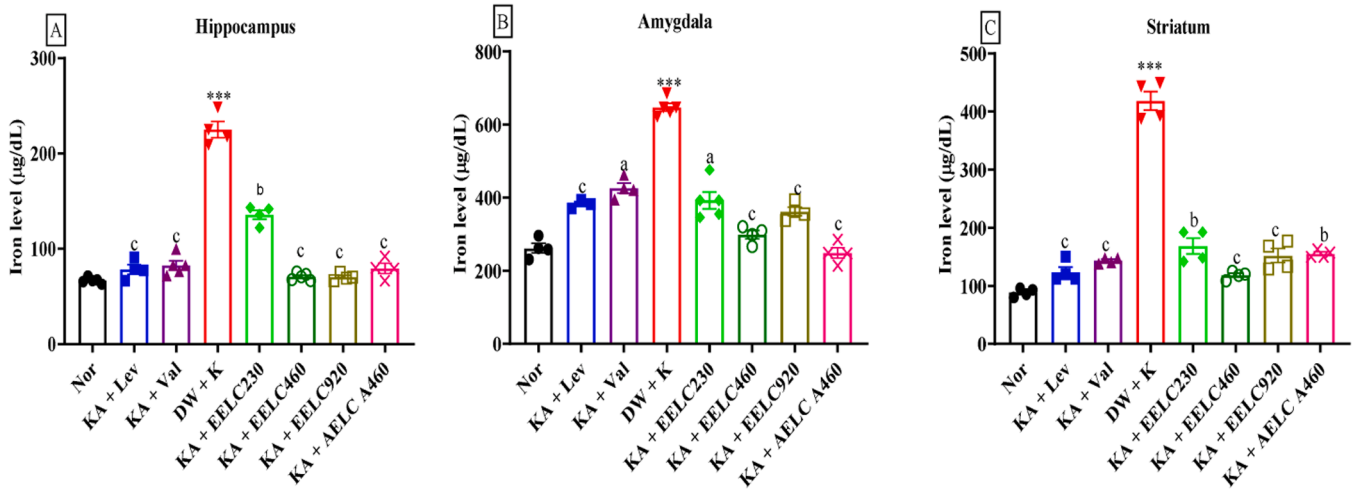

Fig. 9. Effect of the extracts of *L. camara* on iron levels in the hippocampus (A), amygdala (B), and striatum (C). Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs normal control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

normal control and the extract-treated groups, the kainate-treated group showed shrunken and/or pyknotic nuclei and granular cell dispersion (Fig. 12 D1). Also, regions with neuronal loss and a cluster of degenerated neurons (encircled region) were observed in the dentate gyrus of this group (Fig. 12 D2). Fewer malformations are observed in these regions in the extract treatment groups (Fig. 12 E1 and 2, F1 and 2, G1 and 2, and H1 and 2), as well as levetiracetam (Fig. 12 B1 and 2) and valproate-treated groups (Fig. 12 C1 and 2).

Neuronal counting in the CA1 and dentate gyrus regions of the amygdala using ImageJ software revealed a significant increase ($p < 0.001$) in the percentage of degenerating neurons in kainate-treated mice compared to the normal control group (Fig. 12: *lower panel*). These increments were by 192.55 % and 100.56 %, respectively. Compared to the kainate-treated group, treatment with the ethanolic extract (460 mg/kg) of the plant significantly ($p < 0.001$) reduced the percentage of degenerating neurons by 58.36 % and 31.62 %, respectively in the CA1 and dentate gyrus regions. Similarly, treatment with the aqueous extract of the plant significantly ($p < 0.01$) reduced the percentage of degenerating neurons by 48.07 % and 24.36 %, respectively in the CA1 and dentate gyrus regions, compared to kainate-treated mice. Significant reductions ($p < 0.01$) in these numbers were observed in the Levetiracetam-treated group (CA1: 42.48 %; Dentate gyrus: 20.64 %) as well as the valproate-treated group (CA1: 41.75 %; Dentate gyrus: 22.88 %), compared to the kainate group (Fig. 12: *lower panel*).

3.7.2. Effects of the extracts of Lantana camara on the microarchitecture and percentage of degenerating neurons in the amygdala

Microscopic analysis of the basolateral nucleus of the amygdala following hematoxylin and eosin staining revealed several pathological features in kainate-treated mice compared (Fig. 13D, *upper panel*) to the normal control group (Fig. 13A, *upper panel*). These included prominent eosinophilia of the cytoplasm, with some neurons exhibiting karyopyknosis (shrunken nuclei) or pyknosis (condensed and hyperchromatic nuclei). Additionally, kainate treatment induced neuronal swelling and vacuolation (formation of cavities within the cell) (Fig. 13D). Notably, these changes were substantially more evident in the striatum of mice treated with kainate. Furthermore, kainate administration resulted in the development of lipid vacuolation within the brain parenchyma of these mice. Interestingly, the observed pathological changes were completely reversed in mice treated with extracts

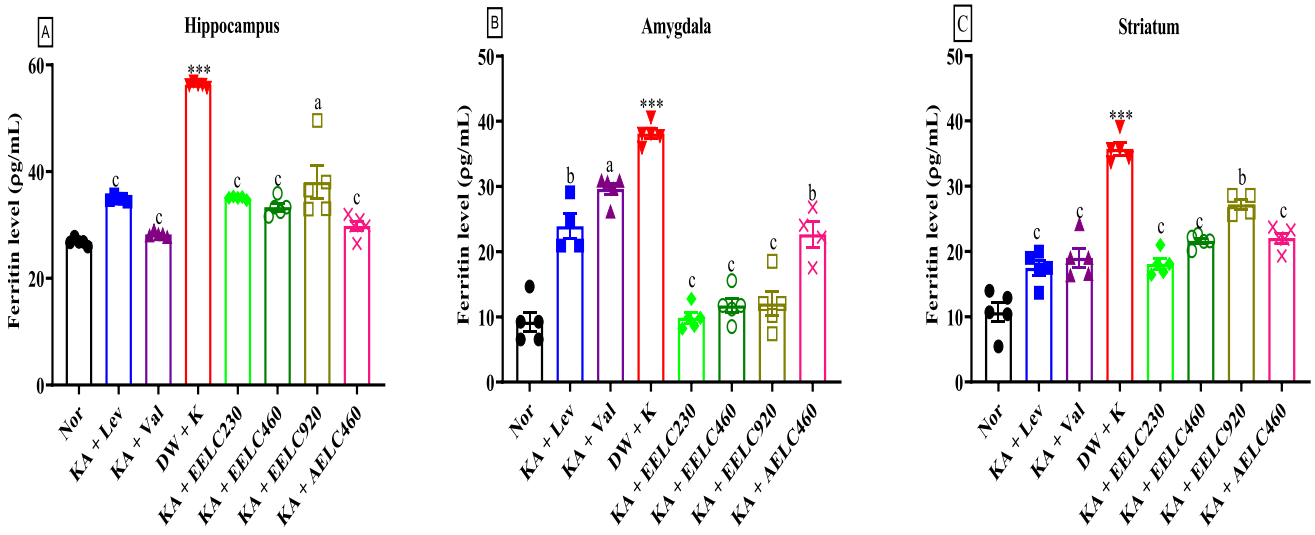

Fig. 10. Effect of the extracts of *L. camara* on ferritin levels in the hippocampus (A), amygdala (B), and striatum (C). Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

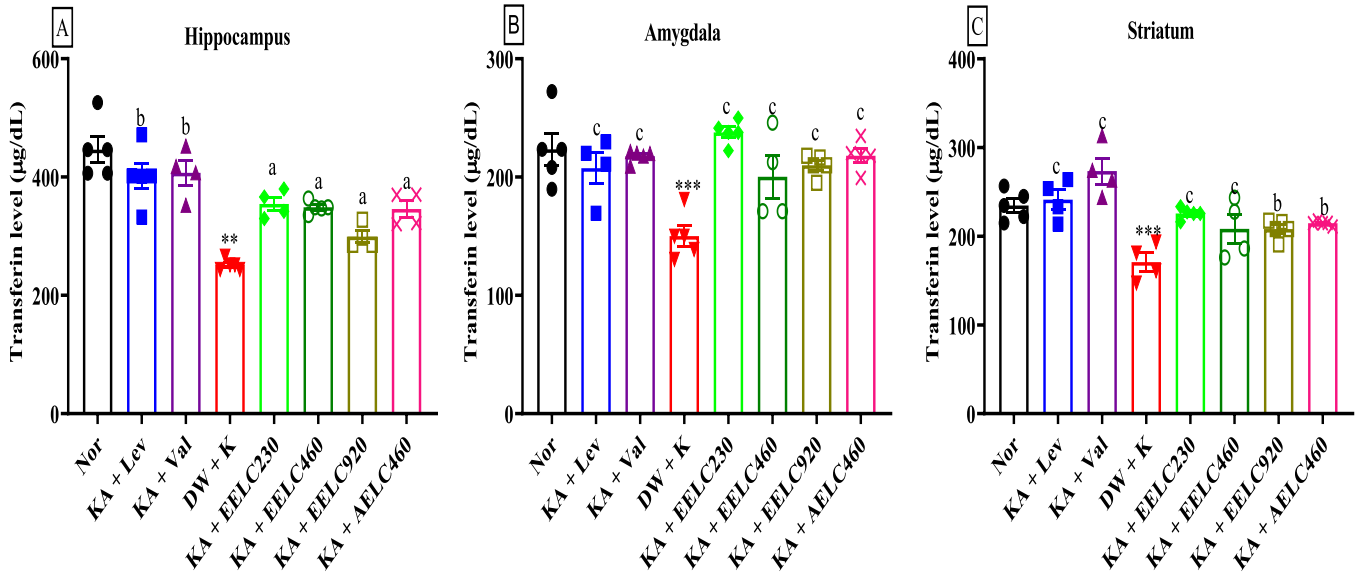

Fig. 11. Effect of the extracts of *L. camara* on transferrin levels in the hippocampus (A), amygdala (B), and striatum(C) Results were expressed as mean ± SEM, N = 7. Data were analyzed by one-way ANOVA, followed by Tukey (HSD). ***$p < 0.001$, vs control group, treated only with saline 0.9 %; a$p < 0.05$, b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

(Fig. 13E, F, G, and H), levetiracetam (Fig. 13B), or valproate (Fig. 13C).

Quantification of neurons in the basolateral nucleus area of the amygdala using ImageJ software revealed a significant increase ($p < 0.001$) in the percentage of degenerating neurons in kainate-treated mice compared to the control group (Fig. 13: *lower panel*). Treatment with the ethanolic extract (230 mg/kg) and the aqueous extract of the plant significantly ($p < 0.001$) reduced the percentage of degenerating neurons by 42.43 % and 41.78 %, respectively, compared to kainate-treated mice. Similarly, levetiracetam and sodium valproate administration resulted in significant reductions ($p < 0.001$) in the percentage of degenerating neurons by 41.12 % and 54.94 %, respectively, compared to the kainate group (Fig. 13: *lower panel*).

3.7.3. Effects of the extracts of Lantana camara on the microarchitecture and percentage of degenerating neurons in the striatum

Hematoxylin and eosin staining of the striatum (Caudate Putamen) revealed various characteristics of degenerating neurons, including significant eosinophilic cytoplasm with or without shrunken nuclei, pyknotic nuclei, and neuron enlargement and vacuolization. These features were more apparent in the striatum of kainate-treated mice (Fig. 14D) than in the normal control group (Fig. 14A). Additionally, kainate treatment caused lipid vacuolation in the brain's parenchyma of these mice (Fig. 14D). The changes were completely reversed in extract-treated mice (Fig. 14E, F, G, and H), as well as those treated with levetiracetam (Fig. 14B) and sodium valproate (Fig. 14C).

Image J neuronal counting of the caudate-putamen revealed an

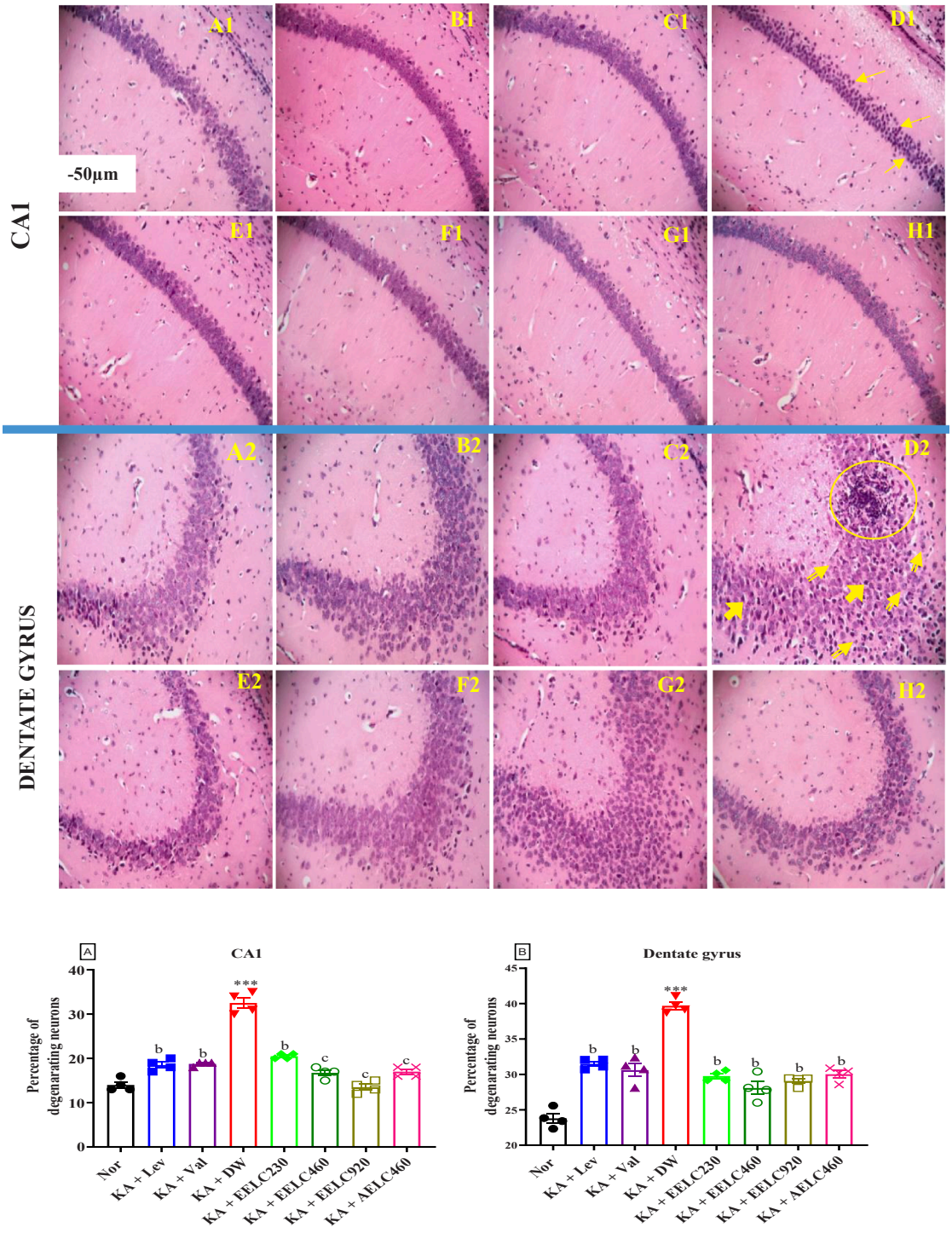

Fig. 12. *Upper panel* – Microphotographs of the *Cornu ammonis* 1 and dentate gyrus after hematoxylin and eosin staining (× 100). DG: dentate gyrus; CA1: *Cornu ammonis* 1; A1 and A2, Normal control groups; B1 and B2, Levetiracetam-treated groups; C1 and C2, Valproate-treated groups; D1 and D2, Kainate-treated groups; E1, F1, G1, and E2, F2, G2, ethanolic extract-treated groups at 230, 460, and 920 mg/kg, respectively; H1 and H2, aqueous extract-treated groups. Normal neurons (stars) can be obtained with rounded nuclei and intact nucleoli. Prominent eosinophilic cytoplasm with or without shrunken nuclei (full arrow), pyknotic nuclei (dash arrows), and neuron swelling and vacuolation (double arrows) are characteristics of degenerating neurons. Scale bar −50 µm. ***Lower panel* - Percentage of degenerating neurons in the *Cornu ammonis* 1 (A) and dentate gyrus (B) after ImageJ neuronal cell count.** Data are mean ± SEM, N = 4 per group. ***$p <$ 0.001, vs control group treated only with saline 0.9 %; b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

increase in the percentage of degenerating neurons in kainate-treated mice by 13.61 %, ($p < 0.001$) relative to the normal control. The ethanolic extract (460 mg/kg) as well as the aqueous extract led to a decrease in the percentage of degenerating neurons by 55.32 %, ($p < 0.001$) and 39.30 %, ($p < 0.001$), respectively when compared to the kainate-treated mice. Levetiracetam as well as sodium valproate led to a decrease in the percentage of degenerating neurons by 42.82 %, ($p < 0.001$) and 35.07 %, ($p < 0.01$), respectively relatively to the kainate-

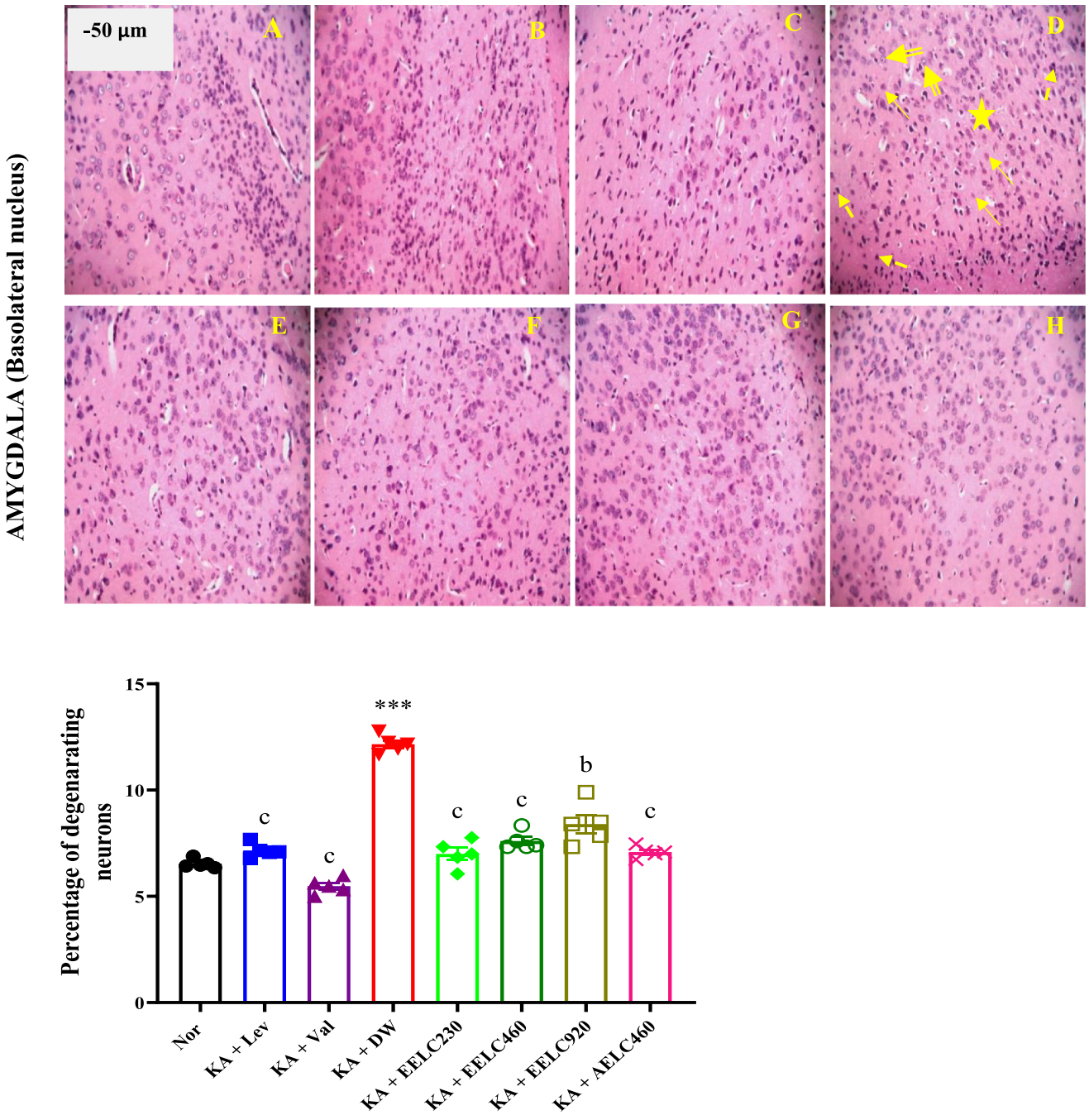

Fig. 13. *Upper panel* – Microphotographs of the amygdala (Basolateral nucleus) after hematoxylin and eosin staining (× 100). A, Normal control; B, Levetiracetam-treated group; C, Valproate-treated group; D, Kainate-treated group; E, F, G, ethanolic extract-treated groups at 230, 460, and 920 mg/kg, respectively; H, aqueous extract-treated group. Normal neurons (stars) can be obtained with rounded nuclei and intact nucleoli. Prominent eosinophilic cytoplasm with or without shrunken nuclei (full arrow), pyknotic nuclei (dash arrows), and neuron swelling and vacuolation (double arrows) are characteristics of degenerating neurons. Scale bar −50 µm. ***Lower panel* - Percentage of degenerating neurons in the amygdala (Basolateral Nucleus) after ImageJ neuronal cell count.** Data are mean ± SEM, N = 4 per group. ***$p < 0.001$, vs control group treated only with saline 0.9 %; b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

treated mice (Fig. 14).

4. Discussion

Kankeda *et al.* conducted significant research on the antiepileptic and anxiolytic properties of an aqueous extract of *Lantana camara* leaves. These authors suggested that antioxidation and GABAergic modulation were potential explanations for the observed effects (Kandeda et al., 2022) Some traditional practitioners treat these ailments with an ethanolic extract of the same plant's leaves. The antiepileptic and anxiolytic properties of this extract have not been studied. The increasing literature also emphasizes the importance of ferroptosis in the development of epilepsy and anxiety disorders. The effect of the two extracts on ferroptosis-related brain indicators has yet to be explored. This research

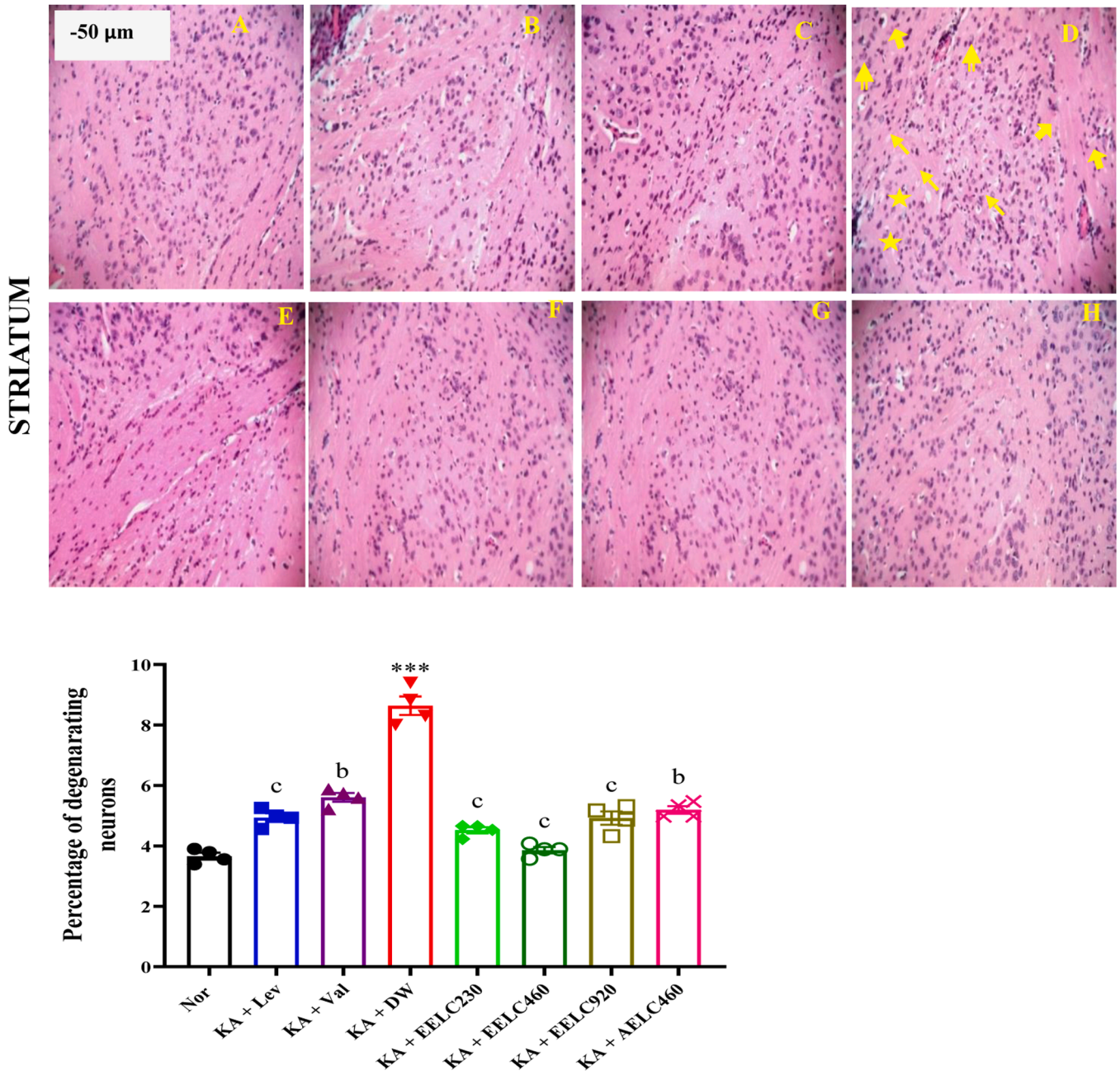

Fig. 14. *Upper panel* – Microphotographs of the striatum (caudate putamen) after hematoxylin and eosin staining (× 100).A, Normal control; B, Levetiracetam-treated group; C, Valproate-treated group; D, Kainate-treated group; E, F, G, ethanolic extract-treated groups at 230, 460, and 920 mg/kg, respectively; H, aqueous extract-treated group. Normal neurons (stars) can be obtained with rounded nuclei and intact nucleoli. Prominent eosinophilic cytoplasm with or without shrunken nuclei (full arrow), pyknotic nuclei (dash arrows), and neuron swelling and vacuolation (double arrows) are characteristics of degenerating neurons. Scale bar −50 µm. ***Lower panel* - Percentage of degenerating neurons in the amygdala (Basolateral Nucleus) after ImageJ neuronal cell count.** Data are mean ± SEM, N = 4 per group. ***$p < 0.001$, vs control group treated only with saline 0.9 %; b$p < 0.01$, c$p < 0.001$, vs kainate group treated with kainate (10 mg/kg) and distilled water (10 ml/kg); LC (230, 460, 920), *L. camara* at the doses of 230, 460, and 920 mg/kg, respectively; KA, kainate; Lev, levetiracetam (80 mg/kg); Val, sodium valproate (300 mg/kg).

delved into the potential benefit of consuming both extracts from *L. camara* for addressing epilepsy and anxiety disorders using kaïnate model. Emphasis was laid on the potentials of extracts of *L. camara* to inhibit ferroptosis.

Kainate is a glutamate analogue and agonist. Neuronal excitotoxicity following glutamate pathway aberrations, seizures and anxiety, ferroptosis, and neurodegeneration are reported features of the kainate model of epilepsy (Lapouble et al., 2006; Scott et al., 2017). In the current study, the number and duration of seizures in mice treated with KA were significantly higher than in the normal control group. These results corroborate those of Kandeda *et al.* and Sedaghat (Kandeda et al., 2022; Sedaghat et al., 2017). These parameters were significantly reversed in the extract-treated groups as well as in both the levetiracetam and valproate treatment groups, comparably to those reported in the KA group. Similar findings were found in several investigations using the kainate model (Xie et al., 2022; Zhang et al., 2022). In these studies, a reduction in the frequency and duration of seizures indicated an alleviation of seizure-like behavior. The results obtained in the current suggests that the ethanolic extract of the plant, similar to the aqueous extract, can reduce seizure-like behavior in mice.

This study used two mazes to investigate anxiety-like behavior in mice. In the elevated zero maze, mice treated with KA exhibited a lower total distance travelled, average speed, number of entries into the closed arm, and percentage of time spent in the open arm compared to the control group. Similar results were obtained by Maaroufi *et al.* and Kandeda *et al.* and suggest anxiety-like behavior (Kandeda et al., 2022; Maaroufi et al., 2009). The propagation of seizures and their deleterious consequences to zones of the brain such as the hippocampus, striatum, and amygdala involved in the control of emotions and anxiety could be responsible for these observed effects (Zimcikova et al., 2017). The treatment with the extracts of the plant led to a notable reversion of the above-assessed parameters. Extract-treated mice in the periphery zone demonstrated positive results in the open field with regard to of distance traveled, number of crossings, number of entrances, percentage of time spent, longest visit, and average duration of visit. In the central section of the maze, positive results were also attained in terms of travel distance, number of entries, latency to the first entry, percentage of time spent, average speed, and latency to the first entry to the food lid zone. According to these findings, the maze's the central zone, which is the most anxiogenic location, has been thoroughly investigated by extract-treated mice. According to emerging literature, anxiolytic actions are suggested by the ease with which animals explore such settings (Prut and Belzung, 2003; Pulga et al., 2012). Similar observations were made in mice treated with valproate, a reference antiepileptic and anxiolytic drug. These results suggest that the plant's extracts might alleviate anxiety disorders.

In our current research, we observed a significant decrease in GABA levels within the striatum, amygdala, and hippocampal regions of KA-treated mice compared to the control group. Loss of inhibitory interneurons during epilepsy leads to a reduction in GABA release and impairments in neuromodulation. This decrease correlates with seizure activity and anxiety-related behaviors, as reported by numerous studies (Vinti et al., 2021; Kandeda et al., 2022; Hingray et al., 2019). Furthermore, emerging evidence suggests that brain iron status plays a crucial role in regulating GABA levels (Ye et al., 2019). Dysregulation of iron levels can result in emotional and psychological disturbances due to its essential role in the proper functioning of GABA shunt enzymes (Maaroufi et al., 2009; Rouault and Cooperman, 2006). This suggests that the aforementioned epileptic and anxiety-like behaviors observed in mice may be attributed to impairments in iron level modulation. The extract-treated groups, as well as positive controls, exhibited elevated GABA levels compared to the KA-treated group. Several studies have proposed that the anti-seizure and anxiolytic-like effects of these extracts may be mediated by an increase in the inhibitory drive provided by GABA. This could explain the observed antiepileptic and anxiolytic effects in the extract-treated groups. Our previous research indicated that the plant's aqueous extract regulates GABA levels by influencing L-GAD activity and GABA transaminase (Kandeda et al., 2022).

Ferroptosis is a kind of iron-dependent programmed cell death characterized by a high production of lipid-based reactive oxygen species, iron overload, and neuronal degeneration. The detrimental relationship between ferroptosis and the KA-induced epilepsy model was established by Cai and Yang, (2021)). Glutamate accumulation is reported to trigger ferroptosis by blocking the glutamate/cysteine antiporter pathway, which is necessary for the synthesis of GSH (Cai and Yang, 2021; Maher et al., 2018). Moreover, emerging studies have shown that the level of GSH and the labile iron pool are intimately linked. Indeed, GSH can retain iron. Thus, the depletion of GSH results in the release and accumulation of iron (Friedmann Angeli et al., 2014; O'keeffe et al., 2021; Chen et al., 2024). Iron is essential for energy metabolism, biosynthesis, neurotransmission, and myelination, as well as for neuronal activity and appropriate physiological function (Rouault and Cooperman, 2006). Indeed, dysregulation and accumulation of iron contribute to the Fenton reaction, which induces the generation of free radicals and other reactive oxygen species contributing to ferroptosis (Cao and Dixon, 2016). Thus, the current study's reported increase in iron levels and decrease in GABA levels is most likely due to a drop in GSH levels in the investigated mouse brain areas. Cai et Yang and Maher observed similar results (Cai and Yang, 2021; Maher et al., 2018). In the meantime, the groups that received the extract saw a considerable increase in GSH levels. Ye et al., Shao et al., Shao et al., and Zhang et al. all published results that were comparable to this one (Ye et al., 2019; Zhang et al., 2022; Shao et al., 2020; Shao et al., 2022). This result suggest that the extracts of the plant shows anti-ferroptotic activities by modulating GSH levels. Additionally, the hippocampus, striatum, and amygdala of extracts-treated mice showed high amounts of transferrin and low levels of ferritin relatively to the KA-treated group. A low amount of the protein ferritin, which stores iron, in the brain is indicative of low availability of iron (Koorts and Viljoen, 2007). The extracellular iron transporter transferrin, on the other hand, is recognized as a critical positive regulator of ferroptotic cell death (Gomme et al., 2005). Increased transferrin availability has been reported as an approach to mitigate iron influx into neuronal cells and preventing ferroptosis from occurring. Indeed, Transferrin has revealed antioxidant properties, iron regulation, and facilitation of drug delivery potentials in a Parkinsonian *subtansia nigra* (Ayton et al., 2016; Daruich et al., 2019). Similar results were obtained in mice treated with sodium valproate. Valproate has revealed potent anti-ferroptotic activities over the years (Li et al., 2020; Kong et al., 2024). Numerous authors revealed similar findings and reported inhibition of ferroptosis (Hou et al., 2016; Gao et al., 2015; Park and Chung, 2019). These findings suggest that the plant's extracts inhibit ferroptosis by its action on transferrin and ferritin.

Iron can cause lipid peroxidation and ferroptosis by attacking polyunsaturated fatty acids in cell membranes. Iron possibly starts non-enzymatic Fenton reactions and can be integrated into enzymes that produce ROS (Friedmann Angeli et al., 2014; Lei et al., 2019). This stimulates the peroxidation of lipids, producing molecules like malondialdehyde (Friedmann Angeli et al., 2014; Lei et al., 2019). In this investigation, the MDA level was considerably higher in the KA-treated group than in the control group. This corroborates accumulating studies (Mao et al., 2019; Cai and Yang, 2021; Fikry et al., 2024). The level of this marker was significantly reduced in the extract-treated groups. Many authors obtained similar results (Zhang et al., 2022; Jia et al., 2020; Hao et al., 2022). This result lend support to the hypothesis that the ethanolic and aqueous extracts of the plant have anti-ferroptotic potentials. Moreover, ferroptosis and lipid peroxidation disrupt normal cell physiology. This in turn increases oxidative stress and aggravates neuronal damage. In our previous study on kainate model of epilepsy, we reported high levels of nitrites and superoxide dismutase indicative of oxidative stress (Kandeda et al., 2022). Iron, by inducing lipid peroxidation and oxidative stress, can lead to neurodegeneration. Ferroptosis is thus characterized by neurodegeneration and neuronal disorganization (Reichert et al., 2020; Ren et al., 2023). Furthermore, KA-induced excitotoxicity and DNA methylation occurring during ferroptosis are responsible for the pyknotic nuclei observed in brains (Dixon et al., 2012). These alterations were observed in the dentate gyrus, CA1, striatum, and amygdala of KA-treated mice after Hematoxylin and eosin stain. Similar results were obtained by numerous authors (Yao et al., 2019; Chen et al., 2021). Fewer of the above-mentioned alterations were observed in the brains of extract-treated mice. Firky et *al.* (Fikry et al., 2024) obtained a similar result. Chen *et al.* also observe neuroprotection was achieved primarily by suppressing neuronal ferroptosis (Chen et al., 2024). This signifies that the extracts effectively protected the brain of mice against ferroptosis-induced hippocampal, amygdala, and striatal alterations. These results might be the explanation for the antiepileptic and anxiolytic effects observed in this study since the neurons of these brain regions play a critical role in the modulation of anxiety and the initiation of seizures.

5. Conclusion

This study is the first to investigate the effects of extracts of an

ethanolic and aqueous extract of *Lantana camara* on a kaïnate model of epilepsy and anxiety disorders with highlights on some ferroptosis markers. It was observed that the extracts: led to a reduction in the number and duration of epileptic seizures; improved anxiety responses in the elevated zero maze and open field; improved GABA levels; reduced iron levels by modulating iron homeostasis; increased GSH levels while decreasing MDA levels; and preserved the micro-architecture of the hippocampus, striatum and amygdala. However, research into the effect of the plant on ferroptosis in epilepsy is still at an early stage. Firstly, the specific molecules responsible for these effects have yet to be identified in terms of type and quantity. Secondly, the effect of the plant extract on other markers of ferroptosis needs to be established. Thirdly, more mechanistic approaches by these molecules against ferroptosis pathways need to be elucidated. Regardless, the results of this study confirm the claims of some traditional healers in Cameroon regarding the use of various extracts of the plant against epilepsy and its comorbidities.

CRediT authorship contribution statement

Symphorien Mabou Talom: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis. **Antoine Kavaye Kandeda:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Xavier Francois Edzoa:** Software, Resources, Investigation. **Gildas Soffo Moffo:** Software, Resources, Investigation, Formal analysis. **Steve Melton Nkegne:** Software, Resources, Investigation, Formal analysis. **Danielle Bilanda:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that studies were conducted in the absence of any commercial, or academic, or financial relationships.

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References

Alva-Díaz, C., Navarro-Flores, A., Rivera-Torrejon, O., Huerta-Rosario, A., Molina, R.A., Velásquez-Rimachi, V., Morán-Mariños, C., Farroñay, C., Pacheco-Mendoza, J., Metcalf, T., 2021. Prevalence and incidence of epilepsy in Latin America and the Caribbean: a systematic review and meta-analysis of population-based studies. J. Epilepsia 62, 984–996.

Vezzani, A., Fujinami, R.S., White, H.S., Preux, P.M., Blumcke, I., Sander, J.W., Loscher, W., 2016. Infections, inflammation and epilepsy. Acta Neuropathol. 131, 211–234.

Formentin, C., Leonardo de Castro Oliveira, D., Matias, L.G., Joaquim, A.F., Tedeschi, H., Ghizoni, E., 2023. Temporopolar Amygdalohippocampectomy for Mesial temporal lobe epilepsy: a two-dimensional anatomical operative video. World Neurosurg. 177, 67.

Prischich, F., De Rinaldis, M., Bruno, F., Egeo, G., Santori, C., Zappaterreno, A., Fattouch, J., Di Bonaventura, C., Bada, J., Russo, G., Pizzuti, A., Cardona, F., Sa'a, Vullo, V., Giallonardo, A.T., D'Erasmo, E., Pelliccia, A., Vanacore, N., 2008. High prevalence of epilepsy in a village in the Littoral Province of Cameroon. Epilepsy Res. 82, 200–210.

Njamnshi, A.K., Angwafor, S.A., Tabah, E.N., Jallon, P., Muna, W.F., 2009. General public knowledge, attitudes, and practices with respect to epilepsy in the Batibo Health District, Cameroon. Epilepsy Behav. 14, 83–88.

Bekele, B.K., Nebieridze, A., Daniel, I.M., Byiringiro, C., Nazir, A., Kibru, E.A., Wojtara, M., Uwishema, O., 2024. Epilepsy in Africa: a multifaceted perspective on diagnosis, treatment, and community support. J. Ann. Med. Surg. 86, 624–627.

Falco-Walter, J., 2020. Epilepsy—definition, classification, pathophysiology, and epidemiology. : Semin. Neurol. Thieme Med. Publ. Inc. 617–623.

Vinti, V., Dell'Isola, G.B., Tascini, G., Mencaroni, E., Cara, G.D., Striano, P., Verrotti, A., 2021. Temporal lobe epilepsy and psychiatric comorbidity. Front. Neurol. 12, 775781.

Lapouble, E., Depaulis, A., Martin, B., 2006. Histological and electrophysiological characterization of the kaïnate model for the mesial temporal lobe epilepsy in different strains of mice. Epilepsies 18, 101–107.

Mojarad, T.B., Roghani, M., 2014. The anticonvulsant and antioxidant effects of Berberine in Kainate-induced temporal lobe epilepsy in rats. Basic Clin. Neurosci. 5, 124–130.

Ye, Q., Zeng, C., Dong, L., Wu, Y., Huang, Q., Wu, Y., 2019. Inhibition of ferroptosis processes ameliorates cognitive impairment in kainic acid-induced temporal lobe epilepsy in rats. Am. J. Transl. Res. 11, 875–884.

Li, Q., Mao, X.-Y., 2019. Baicalein exerts neuroprotective effects in FeCl3-induced posttraumatic epileptic seizures via suppressing ferroptosis. J. Front. Pharmacol. 10, 459526.

Mao, X.Y., Zhou, H.H., Jin, W.L., 2019. Ferroptosis Induction in Pentylenetetrazole Kindling and Pilocarpine-Induced Epileptic Seizures in Mice. Front. Neurosci. 13, 721.

Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., Morrison, B., 3rd, Stockwell, B.R., 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149, 1060–1072.

Perucca, P., Gilliam, F.G., 2012. Adverse effects of antiepileptic drugs. Lancet Neurol. 11, 792–802.

Li, Q., Li, Q.-Q., Jia, J.-N., Liu, Z.-Q., Zhou, H.-H., Jin, W.-L., Mao, X.-Y., 2020. Sodium valproate protects against neuronal ferroptosis in epilepsy via suppressing lysyl oxidase. J. BioRxiv (31), 126227, 2020.05.

Ye, Q., Zeng, C., Dong, L., Wu, Y., Huang, Q., Wu, Y.J., 2019. Inhibition of ferroptosis processes ameliorates cognitive impairment in kainic acid-induced temporal lobe epilepsy in rats. Am. J. Transl. Res. 11, 875.

Xie, R., Zhao, W., Lowe, S., Bentley, R., Hu, G., Mei, H., Jiang, X., Sun, C., Wu, Y., Yueying, L., 2022. Quercetin alleviates kainic acid-induced seizure by inhibiting the Nrf2-mediated ferroptosis pathway. Free Radic. Biol. Med. 191, 212–226.

Cai, Y., Yang, Z., 2021. Ferroptosis and its role in epilepsy. Front. Cell Neurosci. 15, 696889.

Kandeda, A.K., Mabou, S.T., Moutchida, C., 2022. An aqueous extract of *Lantana camara* attenuates seizures, memory impairment, and anxiety in kainate-treated mice: evidence of GABA level, oxidative stress, immune and neuronal loss modulation. Epilepsy Behav. 129, 108611.

Ghisalberti, E.L., 2000. *Lantana camara* L. (Verbenaceae). Fitoterapia 71, 467–486.

Tadesse, E., Engidawork, E., Nedi, T., Mengistu, G., 2017. Evaluation of the anti-diarrheal activity of the aqueous stem extract of Lantana camara Linn (Verbenaceae) in mice. BMC Complement Alter. Med. 17, 190.

Kalita, S., Kumar, G., Loganathan, K., Venkata, K., Rao, B., 2012. A review on medicinal properties of *Lantana camara* Linn. Res. J. Pharm. Technol. 5, 711–715.

Kazmi, I., Rahman, M., Afzal, M., Gupta, G., Saleem, S., Afzal, O., Shaharyar, M.A., Nautiyal, U., Ahmed, S., Anwar, F., 2012. Anti-diabetic potential of ursolic acid stearoyl glucoside: a new triterpenic gycosidic ester from Lantana camara. Fitoterapia 83, 142–146.

Imran Kazmi, Afzal, Ali, M., B, A. Z, Aftab, D., Ahmaol, Firoz, Anwar, 2013. Anxiolytic potential of ursolic acid derivative-a stearoyl glucoside isolated from *Lantana camara* L. (verbanaceae). Asian Pac. J. Trop. Med. 433–437.

Naz, R., Bano, A., 2013. Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara in different solvents. Asian Pac. J. Trop. Dis. 3, 480–486.

M, V.K., P, P.K., N, S., P, S., K, P., 2015. Antihypertensive activity of the ethanolic extract of Lantana carama leaves on high salt loaded wistar albino rats. Pharmacogn. J. 7.

Amoah, V., Atawuchugi, P., Jibira, Y., Tandoh, A., Ossei, P.P.S., Sam, G., Ainooson, G., 2023. Lantana camara leaf extract ameliorates memory deficit and the neuroinflammation associated with scopolamine-induced Alzheimer's-like cognitive impairment in zebrafish and mice. J. Pharm. Biol. 61, 825–838.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electro Clin. Neurophysiol. 32, 281–294.

Tucker, L.B., McCabe, J.T., 2021. Measuring anxiety-like behaviors in rodent models of traumatic brain injury. Front Behav. Neurosci. 15, 682935.

Bronikowski, A., Carter, P., Swallow, J., Girard, I., Rhodes, J., Garland, T., 2001. Open-field behavior of house mice selectively bred for high voluntary wheel-running. Behav. Genet. 31, 309–316.

Rex, A., Voigt, J.P., Voits, M., Fink, H., 1998. Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. Pharm. Biochem Behav. 59, 677–683.

Lowe, S., Francis, P., Procter, A., Palmer, A., Davison, A., Bowen, D.J.B., 1988. Gamma-aminobutyric acid concentration in brain tissue at two stages of Alzheimer's disease. Brain 111, 785–799.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem Biophys. 82, 70–77.

Wilbur, K., Bernheim, F., Shapiro, O., 1949. Determination of lipid peroxidation. Arch. Biochem. Biophys. 24, 305–310.

Survana, K., Layton, C., Brancroft, J., 2019. Brancroft's teory and practice of histological techniques. Elsevier 9.

Scott, A.J., Sharpe, L., Hunt, C., Gandy, M., 2017. Anxiety and depressive disorders in people with epilepsy: a meta-analysis. Epilepsia 58, 973–982.

Sedaghat, R., Taab, Y., Kiasalari, Z., Afshin-Majd, S., Baluchnejadmojarad, T., Roghani, M., 2017. Berberine ameliorates intrahippocampal kainate-induced status epilepticus and consequent epileptogenic process in the rat: underlying mechanisms. Biomed. Pharmacother. 87, 200–208.

Zhang, X., Wu, S., Guo, C., Guo, K., Hu, Z., Peng, J., Zhang, Z., Li, J., 2022. Vitamin E exerts neuroprotective effects in Pentylenetetrazole kindling epilepsy via suppression of ferroptosis. Neurochem Res. 47, 739–747.

Maaroufi, K., Ammari, M., Jeljeli, M., Roy, V., Sakly, M., Abdelmelek, H., 2009. Impairment of emotional behavior and spatial learning in adult Wistar rats by ferrous sulfate. Physiol. Behav. 96, 343–349.

Zimcikova, E., Simko, J., Karesova, I., Kremlacek, J., Malakova, J., 2017. Behavioral effects of antiepileptic drugs in rats: are the effects on mood and behavior detectable in open-field test? Seizure 52, 35–40.
Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. J. Eur. J. Pharmacol. 463, 3–33.
Pulga, A., Ruzza, C., Rizzi, A., Guerrini, R., Calo, G., 2012. Anxiolytic- and panicolytic-like effects of Neuropeptide S in the mouse elevated T-maze. Eur. J. Neurosci. 36, 3531–3537.
Hingray, C., McGonigal, A., Kotwas, I., Micoulaud-Franchi, J.A., 2019. The relationship between epilepsy and anxiety disorders. Curr. Psychiatry Rep. 21, 40.
Ye, Q., Trivedi, M., Zhang, Y., Bohlke, M., Alsulimani, H., Chang, J., Maher, T., Deth, R., Kim, J., 2019. Brain iron loading impairs DNA methylation and alters GABAergic function in mice. FASEB J. 33, 2460–2471.
Rouault, T.A., Cooperman, S., 2006. Brain iron metabolism. Semin. Pediatr. Neurol. 142–148.
Maher, P., van Leyen, K., Dey, P.N., Honrath, B., Dolga, A., Methner, A., 2018. The role of Ca2+ in cell death caused by oxidative glutamate toxicity and ferroptosis. J. Cell Calcium 70, 47–55.
Friedmann Angeli, J.P., Schneider, M., Proneth, B., Tyurina, Y.Y., Tyurin, V.A., Hammond, V.J., Herbach, N., Aichler, M., Walch, A., Eggenhofer, E., Basavarajappa, D., Radmark, O., Kobayashi, S., Seibt, T., Beck, H., Neff, F., Esposito, I., Wanke, R., Forster, H., Yefremova, O., Heinrichmeyer, M., Bornkamm, G.W., Geissler, E.K., Thomas, S.B., Stockwell, B.R., O'Donnell, V.B., Kagan, V.E., Schick, J.A., Conrad, M., 2014. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat. Cell Biol. 16, 1180–1191.
O'keeffe, R., Latunde-Dada, G.O., Chen, Y.-L., Kong, X.L., Cilibrizzi, A., Hider, R.C.J.B., 2021. Glutathione and the intracellular labile heme pool. Biometals 34, 221–228.
Chen, J., Wang, Y., Li, M., Zhu, X., Liu, Z., Chen, Q., Xiong, K., 2024. Netrin-1 alleviates early brain injury by regulating ferroptosis via the PPARγ/Nrf2/GPX4 signaling pathway following subarachnoid hemorrhage. Transl. Stroke Res. 15, 219–237.
Cao, J.Y., Dixon, S.J., 2016. Mechanisms of ferroptosis. Cell Mol. Life Sci. 73, 2195–2209.
Shao, C., Yuan, J., Liu, Y., Qin, Y., Wang, X., Gu, J., Chen, G., Zhang, B., Liu, H.-K., Zhao, J., 2020. Epileptic brain fluorescent imaging reveals apigenin can relieve the myeloperoxidase-mediated oxidative stress and inhibit ferroptosis. J. Proc. Natl. Acad. Sci. 117, 10155–10164.
Shao, J., Bai, Z., Zhang, L., Zhang, F., 2022. Ferrostatin-1 alleviates tissue and cell damage in diabetic retinopathy by improving the antioxidant capacity of the Xc (-)-GPX4 system. Cell Death Discov. 8, 426.
Koorts, A.M., Viljoen, M., 2007. Ferritin and ferritin isoforms I: structure–function relationships, synthesis, degradation and secretion. J. Arch. Physiol. Biochem. 113, 30–54.
Gomme, P.T., McCann, K.B., Bertolini, J., 2005. Transferrin: structure, function and potential therapeutic actions. Drug Discov. Today 10, 267–273.
Ayton, S., Lei, P., Mclean, C., Bush, A.I., Finkelstein, D.I., 2016. Transferrin protects against Parkinsonian neurotoxicity and is deficient in Parkinson's substantia nigra. J. Signal Transduct. Target. Ther. 1, 1–6.
Daruich, A., Le Rouzic, Q., Jonet, L., Naud, M.C., Kowalczuk, L., Pournaras, J.A., Boatright, J.H., Thomas, A., Turck, N., Moulin, A., 2019. Iron is neurotoxic in retinal detachment and transferrin confers neuroprotection. J. Sci. Adv. 5, eaau9940.
Kong, Q., Li, F., Sun, K., Sun, X., Ma, J., 2024. Valproic acid ameliorates cauda equina injury by suppressing HDAC2-mediated ferroptosis. CNS Neurosci. Ther. 30, e14524.
Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M.T., Zeh, H.J., 3rd, Kang, R., Tang, D., 2016. Autophagy promotes ferroptosis by degradation of ferritin. Autophagy 12, 1425–1428.
Gao, M., Monian, P., Quadri, N., Ramasamy, R., Jiang, X., 2015. Glutaminolysis and Transferrin regulate ferroptosis. Mol. Cell 59, 298–308.
Park, E., Chung, S.W., 2019. ROS-mediated autophagy increases intracellular iron levels and ferroptosis by ferritin and transferrin receptor regulation. Cell Death Dis. 10, 822.
Lei, P., Bai, T., Sun, Y., 2019. Mechanisms of ferroptosis and relations with regulated cell death: a review. Front. Physiol. 10, 139.
Fikry, H., Saleh, L.A., Mahmoud, F.A., Gawad, S.A., Abd-Alkhalek, H.A., 2024. CoQ10 targeted hippocampal ferroptosis in a status epilepticus rat model. Cell Tissue Res. 1–27.
Jia, J.N., Yin, X.X., Li, Q., Guan, Q.W., Yang, N., Chen, K.N., Zhou, H.H., Mao, X.Y., 2020. Neuroprotective effects of the anti-cancer drug lapatinib against epileptic seizures via suppressing glutathione Peroxidase 4-dependent ferroptosis. Front. Pharmacol. 11, 601572.
Hao, Y., Ou, Y., Zhang, C., Chen, H., Yue, H., Yang, Z., Zhong, X., Hu, W., Sun, P., 2022. Seratrodast, a thromboxane A2 receptor antagonist, inhibits neuronal ferroptosis by promoting GPX4 expression and suppressing JNK phosphorylation. Brain Res. 1795, 148073.
Reichert, C.O., de Freitas, F.A., Sampaio-Silva, J., Rokita-Rosa, L., Barros, P.L., Levy, D., Bydlowski, S.P., 2020. Ferroptosis mechanisms involved in neurodegenerative diseases. Int J. Mol. Sci. 21, 8765.
Ren, C., Tan, P., Gao, L., Zeng, Y., Hu, S., Chen, C., Tang, N., Chen, Y., Zhang, W., Qin, Y., Zhang, X., Du, S., 2023. Melatonin reduces radiation-induced ferroptosis in hippocampal neurons by activating the PKM2/NRF2/GPX4 signaling pathway. Prog. Neuropsychopharmacol. Biol. Psychiatry 126, 110777.
Yao, P., Chen, Y., Li, Y., Zhang, Y., Qi, H., WJZwzbjjyx, Xu, 2019. Hippocampal neuronal ferroptosis involved in cognitive dysfunction in rats with sepsis-related encephalopathy through the Nrf2/GPX4 signaling pathway. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 31, 1389–1394.
Chen, W., Jiang, L., Hu, Y., Tang, N., Liang, N., Li, X.-F., Chen, Y.-W., Qin, H., Wu, L., 2021. Ferritin reduction is essential for cerebral ischemia-induced hippocampal neuronal death through p53/SLC7A11-mediated ferroptosis. J. Brain Res. 1752, 147216.
Chen, J., Shi, Z., Zhang, C., Xiong, K., Zhao, W., Wang, Y., 2024. Oroxin A alleviates early brain injury after subarachnoid hemorrhage by regulating ferroptosis and neuroinflammation. J. Neuroinflamm. 21, 116.