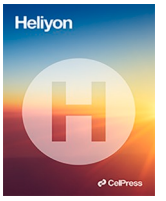
Heliyon 10 (2024) e39483

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Antiepileptic and anti-inflammatory effects of *Lippia multiflora* moldenke (Verbenaceae) in mice model of chronic temporal lobe epilepsy induced by pilocarpine

Joseph Ngaibi [a,*], Bigued [b], Antoine Kavaye Kandeda [c], Yvette Nguezeye [d,e], Tchang Alkali Wangbara [b], Lamido Gaoudji [e], Germain Sotoing Taiwe [f], Elisabeth Ngo Bum [b,e]

[a] *Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon, P.O. Box 67, Dschang, Cameroon*
[b] *Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814, Maroua, Cameroon*
[c] *Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, P.O. Box, Yaounde, Cameroon*
[d] *Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon*
[e] *Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, P.O. Box 454, Ngaoundéré, Cameroon*
[f] *Department of Animal Biology and Conservation, Faculty of Science, University of Buea, Cameroon, P.O. Box 63, Buea, Cameroon*

ARTICLE INFO

Keywords:
Temporal lobe epilepsy
Inflammation
L. multiflora
Epileptogenesis

ABSTRACT

Lippia multiflora Moldenke (Verbenaceae) is an aromatic plant used as a popular medicine with antidepressant, antispasmodic, antifungal, anti-inflammatory, and antioxidant properties. In this study, we explored the effects of *L. multiflora* in mice chronic model of temporal lobe epilepsy induced by pilocarpine and kindled with pentylenetetrazol. Mice were divided into 7 groups of 10 animals, and received a single dose of pilocarpine (360 mg/kg, i.p.), 20 min after the administration of N-methyl-scopolamine (1 mg/kg, i.p). Thirty days after the induction of *status epilepticus*, animals were daily treated for 60 days with distilled water (10 mL/kg, *per os*) for the negative control group, extract (23.07, 57.69, 115.39 and 230.78 mg/kg, *per os*) for the test groups, and sodium valproate (300 mg/kg, i.p) for the positive control group. On every 10th day, animals were injected with a sub-convulsive dose of pentylenetetrazol (15 mg/kg, i.p) 1 h after the administration of the various treatments to assess the susceptibility of animals to seizures. At the end of behavioural tests, animals were sacrificed and the level of inflammatory cytokines was assessed in the hippocampus. The plant extract reduced ($p < 0.001$) the occurrence of seizures and the number of spontaneous recurrent seizures induced by pilocarpine in mice. It ameliorated the levels of inflammatory cytokines (TNF-α, INF- γ, IL-1β, IL-6, and IL-10) in the hippocampus. The *in vitro* studies show that *L. multiflora* have a high amount of total phenolic content, flavonoids and tannins and also have some good antioxidant properties. These results suggest that *L. multiflora* aqueous extracts has the potential to be a promising complementary and alternative medicine for the treatment of epilepsy, due to its antiepileptic, anti-inflammatory and antioxidant effects.

* Corresponding author.
E-mail address: jngaibi@gmail.com (J. Ngaibi).

https://doi.org/10.1016/j.heliyon.2024.e39483
Received 5 May 2024; Received in revised form 12 October 2024; Accepted 15 October 2024
Available online 17 October 2024

1. Introduction

Temporal Lobe Epilepsy (TLE) is the most common form of refractory epileptic disorder often related to childhood seizures [1]. It is the most common and difficult-to-treat type of partial epilepsy. This chronic condition affects 30 million people worldwide, and is characterized by neuronal hyperactivity [2]. Studies in patients have shown that temporal lobe seizures are triggered within the subiculum [3] and the majority of abnormalities observed during TLE in the hippocampus lead to a structural and functional reorganization of its neural network [4,5]. Among others, selective loss of GABAergic interneurons [6] and changes in several $GABA_A$ receptor subunits are also involved in the pathogenesis of TLE [4,7]. An increasing number of experimental evidence suggested a major involvement of inflammation in epileptogenesis [1]. Seizure activity elicits the release of pro-inflammatory cytokines and activates immune responses. Inflammatory processes in the brain can affect the extracellular neuronal matrix integrity. Thus, the prevention of chronic epileptic disorder with an appropriate intervention might represent the most ambitious goal in the clinical treatment [1]. Many drugs used in anticonvulsant therapy exist to control seizures, but drug resistance and side effects are the main limits to these drugs. This explain why researchers are turning toward natural sources for potential remedies. This is the background to our investigation on *Lippia multiflora* Moldenke, a plant of the Verbenaceae family, widely used in the treatment of bronchial ailments, febrile attacks, jaundice, and hypertension [8], coughs and colds [9]. Previous studies showed that the aqueous extract of *L. multiflora* leaves is endowed with analgesic, antipyretic, and anti-inflammatory properties [10]. A number of pharmacological studies also revealed antifungal and antiviral properties of the volatile compounds, as well as antimalarial and diarrheal activity of decoction or infusion of *L. multiflora* [11]. *L. multiflora* is currently attracting increasing interest because of its biomedical virtues [12]. The aim of this study is therefore to assess the potential of *L. multiflora* to alter or inhibit epileptogenesis and inflammatory processes using chronic model of temporal lobe epilepsy in mice.

2. Material and methods

2.1. Biological plant material

The leaves and stems of *L. multiflora* were collected in Ngaoundéré-Dang, locality of Adamawa region of Cameroon. The plant samples has been identified in the national herbarium of Cameroon under number 7753/SRF/Cam. The plant leaves and stems were washed, shade, and air dried, then crushed. The obtained powder was stored at room temperature.

2.2. Preparation of L. multiflora aqueous extracts

One hundred grams of powder were macerated in 1000 mL of distilled water. After 3 h, the macerate was boiled for 20 min. The supernatant was collected, cooled, and filtered with a Whatman number 1 paper. The extract (390 mL) was evaporated *in vacuo* and 9.00 g of dried extract were obtained, and yield 9 %. Knowing the mass of the dry extract and the volume of the decoction obtained, we can determine the concentration of our extract using the following formula: concentration = mass of the dry extract/volume of decocted (concentration = 9000 mg/390 mL = 23.07 mg/mL). The obtained stock solution, or decoction of *L. multiflora* with a concentration of 23.07 mg/mL was administered orally (*per os* (*p.o.*)) to mice in a volume of 10 mL/kg corresponding to a dose of 230.77 mg/kg. This aqueous extract (decoction) was then diluted with distilled water at 1/2, 1/4 and 1/10th and three less concentration solutions (2.307, 5.769, and 11.539 mg/mL) were obtained, respectively.

2.3. Determination of bioactive compounds of L. multiflora

Total phenolic content was evaluated as described by Gao et al. [13] with the Folin-Ciocalteu reagent, the flavonoids content was determined with the aluminium chloride reagent as described by Mimica-Dukic [14], and the tannin content were determined using acidified vanillin [15], respectively.

2.4. In vitro evaluation of the antioxidant activity of L. multiflora aqueous extract

The antioxidant power which is the ability of a given substance to trap a free radical has been determined by Zhang and Hamauzu method [16] with DPPH, and the antioxidant activity was expressed as percent inhibition (% inhibition DPPH = [(Absorbance control – Absorbance sample)/Absorbance control] x 100).

Inhibition of the radical $ABTS^+$ consists in following the kinetics of discoloration of the $ABTS^+$ ion as described by Re et al. [17]. The inhibition percentage was calculated according to the formula: % inhibition ABTS = [(Abs control – Abs sample)/Abs control] x 100.

The reducing power of iron (Fe^{3+}) in the extracts was determined according to the method described by Oyaizu [18]. The results were expressed in milligrams of ascorbic acid equivalent per gram of dry extract.

2.5. Animal and ethical consideration

White mice *Mus musculus* Swiss of both sexes (35 male and 35 female), aged about two months, with a mass of about 20–25 g. They were purchased from the Laboratoire National Veterinaire de Garoua (LANAVET), Cameroon. These mice were raised in the animal

house, and have free access to food and water. All the experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States, National Institutes of Health (NIH publication No. 85–23, revised 1996). This study also received an approval from the University of Buea - Institutional Animal Care and Use Committee (UBIACUC N∘ 008/2019).

2.6. Chemicals

The following reagents were used in this experiment: diazepam from Roche, France; and sodium valproate, N-methyl-scopolamine and pilocarpine hydrochloride were purchased from Sigma Aldrich Inc, St Louis, USA.

2.7. Induction of chronic phase of the temporal lobe epilepsy and study of the antiepileptic property of an aqueous extract of L. multiflora

The mice were randomly divided into 7 groups of 10 mice each, and they were respectively treated with *L. multiflora* aqueous extracts (23.07, 57.69, 115.39 and 230.78 mg/kg, *p.o.*) for the test groups, sodium valproate (300 mg/kg, intraperitoneally (i.p.)) for the positive control group, and distilled water (10 mL/kg, *p.o.*) for negative control group. The normal group of animals received orally distilled water, and injected with saline (0.9 %, i.p.). The injection protocol was similar to those previously described by Turski et *al.* [19], Cavalheiro et *al.* [20], Curia et *al.* [21], and Magnin [22] and was slightly modified. Tonic and clonic seizures, characteristic of the initial state of illness called *status epilepticus* (SE), were induced in mice by intraperitoneal injection of the muscarinic cholinergic agonist pilocarpine, effects that will be able to progress to the chronic phase of temporal lobe epilepsy. On the first day of experiment, a single low dose of N-methyl-scopolamine (1 mg/kg, i.p.) was previously administered to the animals to reduce the cholinergic effects in the peripheral organs initiated by pilocarpine. Twenty minutes after the injection of N-methyl-scopolamine, each animal received a single injection of pilocarpine (360 mg/kg, i.p.) to induce the *status epilepticus*, then they were returned to their respective cages and observed individually for 6 h to determine the severity and duration of acute seizures with reference to the Racine scale [23].

- stage 0: no response;
- stage 1: hyperactivity and vibrissae clonus;
- stage 2: head nodding, head clonus, and myoclonic jerks;
- stage 3: unilateral forelimb clonus;
- stage 4: bilateral forelimb elevation and clonus;
- stage 5: tonic-clonic seizures with loss of righting reflex.

Only mice that developed stage 5 seizures during two consecutive periods were selected for the next steps of study and six animals per group were chosen base on the scale of Racine. Thirty (30) days after the induction of *status epilepticus*, animals were treated daily with distilled water (10 mL/kg) for the negative control group, the different doses of the plant extract for the test groups (23.07, 57.69, 115.39 and 230.78 mg/kg), and sodium valproate (300 mg/kg) for the positive control group. On every 10th day of treatment, each animal receives a sub-convulsive dose of pentylenetetrazol (15 mg/kg, i.p) 1 h after the administration of the various treatments. The procedure of an acute injection of pentylenetetrazol in pilocarpine-treated animals as an important animal model to study the physiological mechanisms underlying pharmaco-resistant epilepsy is to induce spontaneous clonic and tonic convulsions to mice and also to evaluate the efficacy of the plant extract on the triggering flashover of epileptic seizures in the mesial part of the temporal lobe epilepsy [24]. The following behavioural parameters were assessed individually in each mouse for a duration of 30 min: number and duration of clonic or tonic seizures [23,25] every day of treatment. The experiment ended 90 days after the injection of pilocarpine, then 24 h after the study of anticonvulsant properties, i.e. on the 91st day animals were sacrificed. Each animal was euthanized by inhalation of high concentration of compressed carbon dioxide (CO_2) gas in cylinders, and the animal was decapitated. Their brain and then the hippocampus were collected for the evaluation of the biochemical parameters (Fig. 1).

2.8. Determination of the level of inflammatory cytokines in the epileptic animals and treated with an aqueous extract of L. multiflora

The determination of inflammation cytokines was performed by the enzyme-linked immunosorbent assay (ELISA) method using the instruction of the manufacturer. The ELISA kits (Quantikine Colorimetric ELISA Kits (Quantikine®) were obtained from R&D Systems Biotechne, USA.

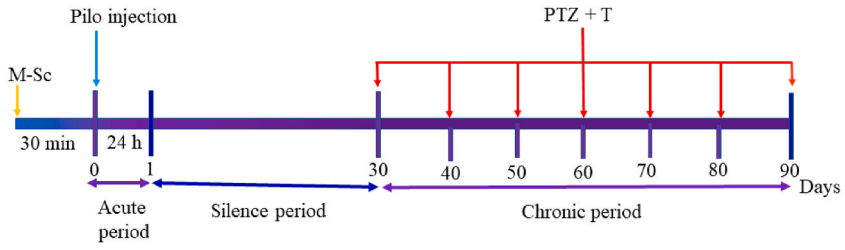


Fig. 1. Work flow for the injection protocol. M − Sc, Methyl-scopolamine; Pilo, pilocarpine; PTZ + T, pentylenetetrazol + treatment.

2.9. Statistical analysis

The results obtained are expressed as means ± standard error on the mean (S.E.M.) or as percentages. Values were compared using the analysis of variance (ANOVA one-way) test and when differences existed, Tukey's multiple comparison tests were used to compare the different groups. At $p < 0.05$, the values were considered significant. The different statistical analyses of the results were done using Graphpad prism version 5.03 software and Microsoft Office Excel 2016.

3. Results

3.1. Effects of L. multiflora aqueous extract on the number of clonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol

Pilocarpine induced the occurrence of spontaneous recurrent seizures characterized by the convulsions in the negative control mice compared to those of normal control animals in which no convulsions were observed. These results indicated that the number of clonic convulsions significantly increased ($p < 0.001$) with a percentage of 63.16 % in period 2 when compared to that of period 1, for the negative control group of mice. The aqueous extract of *L. multiflora* induced a significant reduction of the number of clonic seizures ($p < 0.001$) in a dose-dependent manner. This antiepileptic property is similar to that of sodium valproate (300 mg/kg) were the number of clonic convulsions significantly decreased. At period 5, the number of seizures was at the maximal level in the negative control of mice. However, the aqueous extract significantly reduced [$F(6, 35) = 201.4$; $p < 0.001$] this number with the percentage of reduction of 90.45 % at the dose of 230.78 mg/kg and 96.45 % for the sodium valproate (300 mg kg), respectively. We also noted that at period 6, the number of clonic seizures significantly decreased [$F(6, 35) = 91.41$; $p < 0.001$] in a dose-dependent manner. Between these different studied periods (1–6), there was a significant difference especially in the negative control group of mice when compared with plant extract-treated groups (Fig. 2).

Each bar represents the mean ± SEM of the group, n = 6. $^{a}p<0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; significant difference compared with the normal control (DW + DW) and $^{3}p < 0.001$ compared with the negative control (DW + Pilo); DW: Distilled Water (10 mL/kg); Pilo: pilocarpine (360 mg/kg); L.m: *L. multiflora*; VS: sodium valproate (300 mg/kg).

3.2. Effects of L. multiflora aqueous extract on the duration of clonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol

The intraperitoneal injection of pilocarpine induced a significant increase in the duration of clonic convulsions in the negative control group of mice and which evolved with the number of pentylenetetrazol injection for the respective periods (3–6). The aqueous extract of *L. multiflora* induced a significant reduction in the duration of clonic seizures ($p < 0.001$) in a dose-dependent manner. This antiepileptic property is similar to that of sodium valproate (300 mg/kg) were the duration of clonic convulsions significantly decreased. During the 4th period the results obtained indicated a significant difference between the treated groups of mice [$F(6, 35) = 167.21$; $p < 0.001$], and the treatment of animals with the *L. multiflora* extract induced a significant decrease in a dose-dependent manner in the duration of clonic convulsions, when compared with the negative control. These effects are similar to that of the results obtained during the 5th and 6th periods. Interestingly, the positive control sodium valproate produced the same property in mice when compared with the negative control group (Fig. 3).

Each bar represents the mean ± SEM of the group, n = 6. $^{c}p < 0.001$; significant difference compared with the normal control (DW + DW) and $^{3}p < 0.001$ compared with the negative control (DW + Pilo); DW: Distilled Water (10 mL/kg); Pilo: pilocarpine (360 mg/kg); Lm: *L. multiflora*; VS: sodium valproate (300 mg/kg).

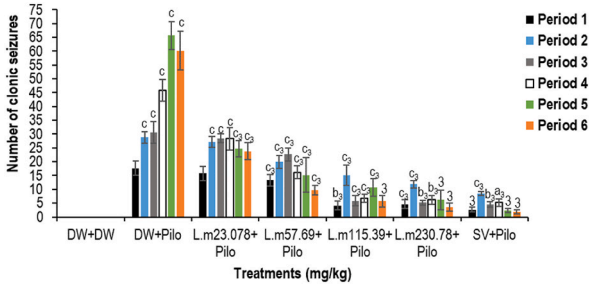


Fig. 2. Effects of L. *multiflora* aqueous extract on the number of clonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol.

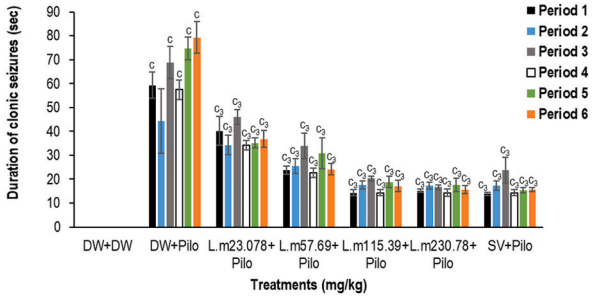


Fig. 3. Effects of L. *multiflora* aqueous extract on the duration of clonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol.

3.3. Effects of L. multiflora aqueous extract on the number of tonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol

The intraperitoneal injection of pilocarpine and the sub-convulsive injection of pentylenetetrazol during the chronic phase of temporal lobe epilepsy within the different studied periods (1–6) induced the occurrence of spontaneous recurrent seizures in mice characterised by tonic convulsions. During the different periods, the appearance of tonic convulsions was observed in the different batches injected with pilocarpine compared to the normal control in which no convulsions were observed. The statistical analysis indicated a significant difference [$F(6, 35) = 45.18$; $p < 0.001$] in the number of clonic convulsions during period 1. It also appears that the number of tonic convulsions were significantly increased during period 2–6. Interestingly, the oral administration of *L. multiflora* aqueous extract induced a significant decrease of the number of tonic convulsions in a dose-dependent manner, when compared with negative control group of mice during the different periods (1–6). The plant extract administered at a doses of 230.78 mg/kg induced a similar effects to that of the reference antiepileptic drug, sodium valproate (300 mg/kg) (Fig. 4).

Each bar represents the mean ± SEM of the group, n = 6. $^{a}p<0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; significant difference compared with normal control (DW + DW) and $^{1}p < 0.05$; $^{2}p < 0.01$; $^{3}p < 0.001$ compared with negative control (DW + Pilo); DW: Distilled Water (10 mL/kg); Pilo: pilocarpine (360 mg/kg); Lm: *L. multiflora*; VS: sodium valproate (300 mg/kg).

3.4. Effects of L. multiflora aqueous extract on the duration of tonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol

The results from Fig. 5 depicted that there is no difference in the duration of tonic convulsions in the negative control group of animals treated with pilocarpine and challenged with a sub-convulsive dose of pentylenetetrazol, for all the different studied periods. However, the administration of *L. multiflora* aqueous extract (230.78 mg/kg) or sodium valproate (300 mg/kg), induced a significant reduction [$F(6, 35) = 60.09$; $p < 0.001$] in the duration of tonic convulsions during the 6th period of study. The plant extracts induced a significant reduction in a dose-dependent manner of this duration when compared with the negative control group of mice. It should be important to indicate that the plant extract administered at a dose of 230.78 mg/kg induced a reduction of 40.92 % in the duration of tonic convulsions during the 2nd period of study, compared with the sodium valproate were the reduction was 38.26 %. At period 6 *L. multiflora* aqueous extract (230.78 mg/kg) or sodium valproate (300 mg/kg) induced 58.93 and 44.78 % reduction in the duration of tonic convulsions when compared with the negative control, respectively.

Each bar represents the mean ± SEM of the group, n = 6. $^{c}p < 0.001$; significant difference compared with normal control (DW + DW) and $^{1}p < 0.05$; $^{2}p < 0.01$; $^{3}p < 0.001$ compared with negative control (DW + Pilo); DW: Distilled Water (10 mL/kg); Pilo: pilocarpine (360 mg/kg); Lm: *L. multiflora*; VS: sodium valproate (300 mg/kg).

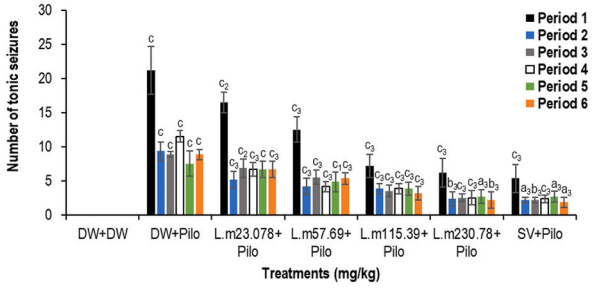


Fig. 4. Effects of L. *multiflora* aqueous extract on the number of tonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol.

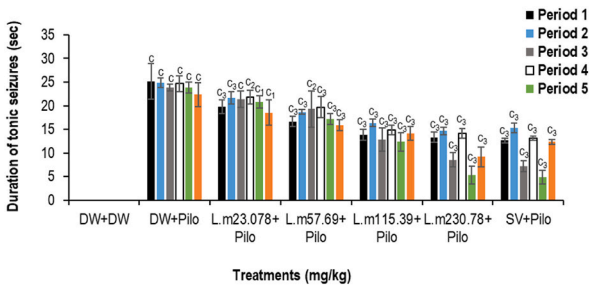


Fig. 5. Effects of L. *multiflora* aqueous extract on the duration of tonic seizures during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol.

3.5. Effects of L. multiflora aqueous extract on the concentration of inflammatory cytokines during the chronic phase of temporal lobe epilepsy induced by pilocarpine and challenged with pentylenetetrazol

The induction of temporal lobe epilepsy by the intraperitoneal injection of pilocarpine in mice resulted in the increase of inflammatory cytokine (TNF-α, INF-γ, IL-1β, IL-6, and IL-10) in hippocampus of distilled water-treated pilocarpine group of animals when compared with the normal group treated with distilled wated and injected with saline. The oral administration of *L. multiflora* aqueous extract to epileptic mice significantly ameliorated the level of inflammatory cytokines by decreasing ($p < 0.001$) the concentration TNF-α, INF-γ, IL-1β, IL-6, and IL-10 when compared with the negative control group. In addition, the same effect was observed in the concentrations of inflammatory cytokines measured in the positive control group of mice treated with sodium valproate. The effects of the plant extract were comparable for the doses of 115.39 and 230.78 mg/kg, as well as for the sodium valproate (300 mg/kg) (Table 1).

Each value represents the mean ± MSE of the group, n = 6. $^{c}p < 0.001$; significant difference compared to normal control (DW + DW) and $3p < 0.001$ compared to negative control (DW + Pilo). DW: Distilled water (10 mL/kg); Pilo: Pilocarpine; Lm: *L. multiflora*; VS: sodium valproate (positive control). TNF: tumor necrosis factor; INF: interferon; IL: interleukin.

3.6. Bioactive compounds of L. multiflora aqueous extract and In vitro antioxidant activity

L. multiflora aqueous extract has a significantly high contents of polyphenol (163.55 ± 0.01 mg GAE/g extract) and tannin content (14.27 ± 0.05 mg TAE/g extract) per gram of dry extract. But the total flavonoid content (13.78 ± 0.01 mg Rutin/g extract) is not as much (Table 2).

The ferrous reducing antioxidant power of the *L. multiflora* aqueous extract was 44.52 ± 0.02 mg AAE/g extract. The percentage inhibition of DPPH and ABTS increases significantly in a dose-dependent manner. IC50 is comparable to that of vitamin C, the reference antioxidant (Table 2).

Each value is expressed as mean ± SEM, n = 5. Vit C: Vitamin C, IC50: Inhibitory concentration 50.

4. Discussion

Administration of pilocarpine induced spontaneous recurrent seizures in mice treated with distilled water during the chronic phase of temporal lobe epilepsy. It induces seizures by stimulating massive exocytosis of L-glutamate in the synaptic cleft [26]. Indeed, pilocarpine can act on both M1 and M2 muscarinic receptors. However, upon binding to the M1 receptor, pilocarpine activates phospholipase C and produces diacylglycerol and inositol triphosphate, which result in altered Ca^{2+} and K^{+} ion currents and increase brain excitability [27]. This increased excitability is due to reduced ATPase activity in the hippocampus, which could not repolarize the plasma membrane or calcium extrusion [28]. In the hippocampus of pilocarpine-treated animals, Na^{+}/K^{+} ATPase activity is modified [29]. Its activity is reduced during the acute and silent period and an increased activity during the chronic phase, showing that the hippocampus of these animals also has an ionic imbalance related to its maintained excitability [29]. This imbalance comes from the increase in Ca^{2+} concentration leading to an increase in glutamate release that induces SE and is probably responsible for the

Table 1
Effects of L. *multiflora* on markers of inflammation during the chronic phase.

Treatments	Doses (mg/kg)	TNF-α (pg/mL)	INF-δ (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
DW + DW	– + –	153.49 ± 21.42	151.05 ± 11.62	150.80 ± 7.42	320.04 ± 11.60	149.88 ± 7.66
DW + Pilo	– + 360	345.73 ± 15.32^{c}	381.37 ± 6.54^{c}	329.93 ± 19.32^{c}	485.86 ± 11.47^{c}	327.18 ± 19.32^{c}
Lm + Pilo	23.078 + 360	228.22 ± 14.49^{c3}	283.83 ± 4.99^{c3}	252.68 ± 6.87^{c3}	361.65 ± 23.38^{c3}	250.48 ± 6.68^{c3}
Lm + Pilo	57.69 + 360	198.00 ± 5.43^{c3}	229.13 ± 17.98^{c3}	212.95 ± 24,91^{c3}	324.31 ± 2.04^{3}	169.09 ± 21.62^{3}
Lm + Pilo	115.39 + 360	144.01 ± 3.31^{3}	158.09 ± 13.18^{3}	166.96 ± 12.31^{3}	328.43 ± 12.74^{3}	156.43 ± 15.16^{3}
Lm + Pilo	230.78 + 360	151.22 ± 5.93^{3}	144.01 ± 14.90^{3}	142.15 ± 10.28^{3}	314.23 ± 5.67^{3}	143.03 ± 8.50^{3}
VS + Pilo	300 + 360	154.78 ± 4.99^{3}	159.88 ± 12.69^{3}	149.59 ± 7.50^{3}	316.10 ± 5.28^{3}	147.91 ± 7.28^{3}

Table 2
Percentage of inhibition of DPPH and ABTS, and IC50 values.

	% Inhibition of DPPH			% Inhibition of ABTS	
Concentration (mg/mL)	*L. multiflora*	Vit C	Concentration (mg/mL)	*L. multiflora*	Vit C
0.1	64.43 ± 0.03	82.34 ± 0.00	0.025	26.86 ± 0.00	80.41 ± 0.00
0.25	78.38 ± 0.02	86.93 ± 0.00	0.05	32.91 ± 0.00	84.58 ± 0.00
0.5	88.15 ± 0.01	96.36 ± 0.00	0.1	49.74 ± 0.00	91.98 ± 0.00
1	96.18 ± 0.00	98.44 ± 0.00	0.25	78.59 ± 0.01	94.07 ± 0.00
IC50	**0.059 ± 0.01**	**0.019 ± 0.00**	**IC50**	**0.102 ± 0.00**	**0.061 ± 0.00**

appearance of spontaneous seizures [29]. After the induction of *status epilepticus*, the silent phase or epileptogenesis follows, which represents the time interval between the initial state and the onset of spontaneous recurrent seizures or ictogenesis, during which synaptic reorganization and active budding of mossy fibres are observed. The resulting seizures are a consequence of the ongoing synaptic reorganization [30]. Cavalheiro et *al.* [30] suggested that structural brain damage caused by pilocarpine-induced *status epilepticus* results in spontaneous recurrent seizures in mice with characteristics similar to those described in humans with temporal lobe epilepsy. The onset of spontaneous recurrent seizures after pilocarpine injection leads to neuronal loss in the hilum of the dentate gyrus and the CA1 and CA3 layers of the hippocampal formation, as well as proliferation of astrocytes [30]. Results showed in this study indicated that the treatment with *L. multiflora* aqueous extract reduced seizure activity and decreased the number of spontaneous recurrent seizures in mice. This would be due to an inhibition of Ca^{2+} exocytosis caused by glutamate. The inhibition of this exocytosis results in the cessation of seizures [21]. The results obtained also show that *L. multiflora* aqueous extract decrease the number and duration of clonic and tonic convulsions that would be due to the presence of certain compounds (polyphenols, tannins, flavonoids) in the extract that would antagonize the effects of pilocarpine and potentiate the GABAergic transmission. Indeed, Zhu et *al.* [31] proved that compounds such as alkaloids, flavonoids, saponins, terpenoids and coumarins have anticonvulsant or antiepileptogenic activity. In addition, flavonoids act on modulation of the GABAergic system and reduction of neuronal excitability by blocking voltage-dependent Na^{+} channels as well as on the GABAergic system by modulating its effects [31] and coumarins, on $GABA_A$ receptors by increasing chlorine current conductance. Previous studies on saponins isolated from the plant extracts have proved their anticonvulsant potential due to blocking voltage-gated sodium channels [32]. It has been reported that saponins and phenolic compounds possess inhibitory action on different types of Ca^{2+} channels, causing the reduction of their opening time, prolonging their closing time and reducing their open state probability [33,34]. Glutamatergic functions are also inhibited by saponin treatment, causing blockage of NMDA receptors responsible for excitatory processes in rat hippocampal cells [33]. Flavonoids act on the $GABA_A$ receptor complex while alkaloids interact with the voltage-dependent Na^{+} channel causing a blockade, responsible for the reduction of neuronal excitability.

In inflammatory conditions, damage to the blood barriers brain facilitates the entry of circulating monocytes into the CNS, a small number of which differentiate into microglia [35]. These cells will promote the release of inflammatory factors such as interleukin-1, TNF-alpha (tumor necrosis factor), cyclooxygenase-2, complement factors, chemokines (or chemokines). In the case of epilepsy, the hypothesis is that neuronal hyperexcitability may cause a release of these mediators and thus be at the origin of a pro-inflammatory reaction leading to an abnormality of the blood-brain barrier, which in turn, would aggravate the neuronal hyperexcitability. Inflammatory factors may therefore contribute to the generation of seizures, as well as participate in the development of epileptogenesis and pharmaco-resistance in animal models [36–38]. Activation of TLR_4 receptors by their endogenous ligand will lead to increased Ca^{2+} inputs into the cell through phosphorylation of the NR2B subunit of the NMDA receptor, which will increase cellular excitability [39]. This explains the increase in TNF-α, INF-γ, IL-6, IL-1β and IL-10 in negative control animals. The more proinflammatory cytokines are produced, the more the body responds by releasing anti-inflammatory cytokines, and the less inflammation factors are released, the less the body releases anti-inflammatory molecules. This explains the increase in IL-10 levels in the negative control group and its decrease in the groups treated with aqueous extract of *L. multiflora* and with sodium valproate and diazepam [40]. The results obtained showed that the treatment with the *L. multiflora* aqueous extract reduced the levels of cytokines in the hippocampus. The treated mice showed significantly lower levels of inflammatory cytokines when compared with the negative control group. This ameliorative property could be due to the blockage of the synthesis of these respective factors by microglia, an action that would be attributed to IL-10. IL-10 is an anti-inflammatory cytokine with important roles in preventing inflammation [37,40]. It regulates the exuberant immune response of microglia by inhibiting the release of pro-inflammatory mediators such as TNF-α and IL-1β [37]. According to the studies conducted, the immunosuppressive effects of IL-10 is to block the synthesis of pro-inflammatory cytokines in T cells, macrophages and microglial cells [41]. Furthermore, IL-10 induces the synthesis of the endogenous IL-1β antagonist (IL-1Ra) as well as the soluble form of the TNF-α receptor to limit the action of these pro-inflammatory cytokines by another pathways [41,42]. *L. multiflora* aqueous extract could act as an IL-10 receptor agonist to inhibit microglial proliferation and reactive astroglial reduction. Its anti-inflammatory effect would be attributed to its action on the reduction of recurrent seizures induced by pilocarpine. These effects are due to the presence of certain compounds in the extract of *L. multiflora* such as flavonoids, alkaloids and polyphenols, and their antioxidant capacity. These results are supported by Jigam et *al* [43] who showed that the significant analgesic and anti-inflammatory effects were attributed to the presence of alkaloids and flavonoids. In addition, the work of Lima et *al* [43] and Pina et *al* [44] showed that some of the most important monoterpenes found in the essential oil of *L. multiflora* inhibit allergic inflammation through modulation of inflammatory cytokines. The results obtained in the phytochemical studies could support the anti-inflammatory and antiepileptic effects of the plant extract.

5. Conclusion

This study demonstrated that the administration of *L. multiflora* had an ameliorative effects on a mice model of chronic temporal lobe epilepsy induced by pilocarpine injection and challenged with pentylenetetrazol. The results showed that *L. multiflora* aqueous extract reduced the number and duration of epileptic convulsions, the levels of inflammatory cytokines. These findings could justify the use of *L. multiflora* aqueous extracts in by the traditional healers for the treatment of temporal lobe epilepsy, the pharmaco-resistant type of epilepsy. Further studies are necessary to evaluate the efficacy and safety of *L. multiflora* in the mice model of generalised clonic-tonic convulsions in mice.

CRediT authorship contribution statement

Joseph Ngaibi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bigued:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Antoine Kavaye Kandeda:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Yvette Nguezeye:** Visualization, Validation, Project administration, Methodology, Data curation. **Tchang Alkali Wangbara:** Visualization, Investigation, Data curation, Conceptualization. **Lamido Gaoudji:** Visualization, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Germain Sotoing Taiwe:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elisabeth Ngo Bum:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition.

Statement of ethical approval

The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85–23, revised 1996) and the Cameroon National Ethical Committee, Yaounde (No. FW-IRB00001954).

Data availability

Data will be made available on request.

Declaration of competing interest

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

Acknowledgments

The authors are very thankful to LabEx Physiology, Pharmacological Targets and Therapeutics, University of Buea, Cameroon, for supporting us by providing apparatus and drugs.

References

[1] R. Di Maio, Neuronal Mechanisms of Epileptogenesis, Frontiers Media SA, 2014, p. 29.
[2] A.V. Petersen, J.-F. Perrier, La sérotonine prévient l'épilepsie du lobe temporal en inhibant les neurones à bouffées du subiculum, M-S (Med. Sci.) 33 (8–9) (2017) 727–729.
[3] I. Cohen, et al., On the origin of interictal activity in human temporal lobe epilepsy in vitro, Science 298 (5597) (2002) 1418–1421.
[4] M. Avoli, et al., Cellular and molecular mechanisms of epilepsy in the human brain, Progress in neurobiology 77 (3) (2005) 166–200.
[5] P. Andersen, et al., The hippocampus Book, Oxford university press, 2006.
[6] R. Cossart, et al., Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy, Nat. Neurosci. 4 (1) (2001) 52–62.
[7] C.R. Houser, M. Esclapez, Downregulation of the α5 subunit of the GABAA receptor in the pilocarpine model of temporal lobe epilepsy, Hippocampus 13 (5) (2003) 633–645.
[8] B. Bayala, Etude des propriétés anti-oxydantes, anti-inflammatoires, anti-prolifératives et anti-migratoires des huiles essentielles de quelques plantes médicinales du Burkina Faso sur des lignées cellulaires du cancer de la prostate et de glioblastomes, Université Blaise Pascal-Clermont-Ferrand II; Université Joseph Ki-Zerbo (2014).
[9] P. Rovesti, M. Bissangou, J. Ouamba, Valorisation chimique de quelques espèces aromatiques et médicinales du congo, Ageratum conyzoides, 1997, pp. 70–80.
[10] A. Abena, et al., Analgesic, antipyretic and anti-inflammatory effects of essential oil of Lippia multiflora, Fitoterapia 74 (3) (2003) 231–236.
[11] K. Oussou, et al., Etude chimique et activité antidiarrheique des huiles essentielles de deux plantes aromatiques de la pharmacopée ivoirienne, Eur. J. Sci. Res. 24 (1) (2008) 94–103.
[12] L.B. Diomande, et al., Occurrence et composition chimique de l'huile essentielle des feuilles de Lippia multiflora M.(thé de savane) selon le pH, les teneurs en Carbone, en Azote et Phosphore du sol en zones de savane guinéenne en Côte d'Ivoire, Afr. Sci. Rev. Int. Sci. Technol. 10 (4) (2014) 93–108.

[13] X. Gao, et al., Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation, J. Agric. Food Chem. 48 (5) (2000) 1485–1490.
[14] N. Mimica-Dukic, Investigation on Secondary Biomolecules in Some Mentha-Species, University of Novi Sad, 1992. Thesis.
[15] Z. Bainbridge, et al., Methods for assessing quality characteristics of non-grain starch staples.(Part 3. Laboratory methods.) (1996).
[16] D. Zhang, Y. Hamauzu, Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking, Food Chem. 88 (4) (2004) 503–509.
[17] R. Re, et al., Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free radical biology and medicine 26 (9–10) (1999) 1231–1237.
[18] M. Oyaizu, Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine, The Japanese journal of nutrition and dietetics 44 (6) (1986) 307–315.
[19] W.A. Turski, et al., Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological study, Behav. Brain Res. 9 (3) (1983) 315–335.
[20] E. Cavalheiro, et al., Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous I recurrent seizures, Epilepsia 32 (6) (1991) 778–782.
[21] G. Curia, et al., The pilocarpine model of temporal lobe epilepsy, J. Neurosci. Methods 172 (2) (2008) 143–157.
[22] E. Magnin, Étude de l'expression de la sous-unité alpha 5 des récepteurs GABAA dans les interneurones de l'hippocampe et de sa diminution dans l'épilepsie du lobe temporal (2014).
[23] R.J. Racine, Modification of seizure activity by electrical stimulation: II. Motor seizure, Electroencephalogr. Clin. Neurophysiol. 32 (3) (1972) 281–294.
[24] M.M. Blanco, et al., Assessment of seizure susceptibility in pilocarpine epileptic and nonepileptic Wistar rats and of seizure reinduction with pentylenetetrazole and electroshock models, Epilepsia 50 (4) (2009) 824–831.
[25] G. Taiwe, et al., Effects of a lyophilized aqueous extract of Feretia apodanthera Del.(Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice, Epilepsy Behav. 43 (2015) 100–108.
[26] T. Lemos, E.A. Cavalheiro, Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats, Exp. Brain Res. 102 (1995) 423–428.
[27] M. Segal, Synaptic activation of a cholinergic receptor in rat hippocampus, Brain Res. 452 (1–2) (1988) 79–86.
[28] M.G. Funke, et al., Calcium homeostasis and temporal lobe epilepsy, ARQUIVOS DE NEUROPSIQUIATRIA 61 (2003) 8–14.
[29] F.A. Scorza, et al., The pilocarpine model of epilepsy: what have we learned? An. Acad. Bras. Cienc. 81 (2009) 345–365.
[30] E. Cavalheiro, N. Santos, M. Priel, The pilocarpine model of epilepsy in mice, Epilepsia 37 (10) (1996) 1015–1019.
[31] H.L. Zhu, et al., Medicinal compounds with antiepileptic/anticonvulsant activities, Epilepsia 55 (1) (2014) 3–16.
[32] D. Liu, et al., Voltage-dependent inhibition of brain Na+ channels by American ginseng, Eur. J. Pharmacol. 413 (1) (2001) 47–54.
[33] H.-J. Kim, et al., Involvement of endogenous prostaglandin F2α on kainic acid-induced seizure activity through FP receptor: the mechanism of proconvulsant effects of COX-2 inhibitors, Brain Res. 1193 (2008) 153–161.
[34] G. Zhong, et al., Calcium channel blockade and anti-free-radical actions of panaxadiol saponins Rb1, Rb2, Rb3, Rc, and Rd, Zhongguo yao li xue bao= Acta Pharmacologica Sinica 16 (3) (1995) 255–260.
[35] A. Mildner, et al., Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions, Nat. Neurosci. 10 (12) (2007) 1544–1553.
[36] E. Croisier, M.B. Graeber, Glial degeneration and reactive gliosis in alpha-synucleinopathies: the emerging concept of primary gliodegeneration, Acta Neuropathol. 112 (2006) 517–530.
[37] A. Vezzani, et al., The role of inflammation in epilepsy, Nat. Rev. Neurol. 7 (1) (2011) 31–40.
[38] G. Terrone, et al., Preventing epileptogenesis: a realistic goal? Pharmacol. Res. 110 (2016) 96–100.
[39] W. Ouyang, et al., Regulation and functions of the IL-10 family of cytokines in inflammation and disease, Annu. Rev. Immunol. 29 (1) (2011) 71–109.
[40] Z. He, et al., Intrathecal lentivirus-mediated transfer of interleukin-10 attenuates chronic constriction injury-induced neuropathic pain through modulation of spinal high-mobility group box 1 in rats, Pain Physician 16 (5) (2013) E615.
[41] J.J. Haddad, N.E. Saadé, B. Safieh-Garabedian, Interleukin-10 and the regulation of mitogen-activated protein kinases: are these signalling modules targets for the anti-inflammatory action of this cytokine? Cell. Signal. 15 (3) (2003) 255–267.
[42] D.A. Joyce, et al., Two inhibitors of pro-inflammatory cytokine release, interleukin-10 and interleukin-4, have contrasting effects on release of soluble p75 tumor necrosis factor receptor by cultured monocytes, Eur. J. Immunol. 24 (11) (1994) 2699–2705.
[43] A.A. Jigam, et al., In vivo antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of Lippia multiflora mold (2009).
[44] L.T. Pina, et al., Alcoholic monoterpenes found in essential oil of aromatic spices reduce allergic inflammation by the modulation of inflammatory cytokines, Nat. Prod. Res. 33 (12) (2019) 1773–1777.