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Anticonvulsant properties of an aqueous extract of *Dysphania ambrosioides* (L.) Mosyakin and Clemants (Chenopodiaceae) in mice pilocarpine model of temporal lobe epilepsy

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Abstract

Ethnopharmacological relevance: *Dysphania ambrosioides* (L.) Mosyakin and Clemants (Chenopodiaceae) is used in traditional Cameroonian medicine to treat epilepsy and anxiety.

Aims of the study: This study aimed to investigate the anticonvulsant effects of *Dysphania ambrosioides* aqueous extract in mice pilocarpine model of temporal lobe epilepsy.

Materials and Methods: The mice were treatments with distilled water for the normal and negative control, sodium valproate (300 mg/kg) for the positive control, and different doses of an aqueous extract of *Dysphania ambrosioides (*37, 92.5, 185, 370 mg/kg, *p.o*) for the test groups. Methyl-scopolamine (1 mg/kg) were injected forty minute later. One hour after the first treatment (first day), epilepsy was induced by intraperitoneal injection of 360 mg/kg pilocarpine. On the seventh day, antioxidant activities and the involvement of GABAergic transmission were determined by measuring the levels of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), γ-Aminobutyric acid (GABA) and GABA-transaminase (GABA-T), respectively in the hippocampus of sacrificed epileptic mice.

Results: *Dysphania ambrosioides* (370 mg/kg) strongly protected mice against epileptogenesis by increasing the latency time to *status epilepticus* ($p<0.001$) and decreasing the number of tonic clonic convulsions ($p<0.001$). The extract significantly increased the levels of GSH ($p<0.001$), CAT ($p<0.001$), SOD ($p<0.001$) and GABA ($p<0.001$), and decreased the levels of MDA ($p<0.001$), NO ($p<0.001$) and GABA-T ($p<0.001$).

Conclusion: The results suggest that the anticonvulsant activities of *Dysphania ambrosioides* extract are accompanied by its antioxidant effects, and may be mediated at least in part by the GABA neurotransmission.

Keywords: Anticonvulsant; Temporal Lobe Epilepsy; Γ-Aminobutyric Acid; Oxidative Stress; *Dysphania Ambrosioides*.

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1. Introduction

Mesial temporal lobe epilepsy (MTLE) represents the most common form of refractory epilepsy. The hippocampus and para-hippocampus appear to be key structures involved in epilepsy [1, 2]. In the preoperative assessment of patients with drug-resistant MTLE, 70% have hippocampal atrophy detected on magnetic resonance imaging [3]. In operated patients, 70% also have hippocampal sclerosis [4]. In Cameroon, MTLE represents 10% of forms of epilepsy [5]. Despite effective treatments for this form of epilepsy, approximately two-thirds of patients suffering from MTLE are resistant to current pharmacotherapies [6, 7]. Furthermore, the adverse effects associated with antiepileptic drugs and recurrent seizures limit their use [8, 9]. The search for new therapeutic agents continues, and medicinal plants are crucial source for the development of drugs to treat neurological disorders and play an important role for patients who respond poorly to conventional treatments [9, 10]. Resistance to pharmacological treatment may be the consequence of progressive ineffectiveness of inhibition by the gamma-aminobutyric acid (GABA) system due to internalization of receptors [11] and activation of glutamate receptors, particularly N-methyl-D-aspartate (NMDA) receptors. Oxidative stress plays a role in the initiation and progression of epilepsy, and therapies aimed at reducing oxidative stress may ameliorate tissue damage and favourably modify the clinical course [12, 13]. Recognition of the relationship between oxidative stress and neuronal loss in epilepsy has sparked intense interest in the development of antioxidant molecules to protect neurons from oxidation-related damage following a seizure [13, 14]. It is therefore important to look for anticonvulsants with antioxidant properties.

Dysphania ambrosioides (L.) Mosyakin and Clemants (Chenopodiaceae) is a traditional aromatic herbal medicine used to treat neuropathic pain, inflammatory conditions such as cholecystitis, arthritis, and gastritis as well as neuropsychiatric disorders [15]. The decoction prepared from the aerial part of *Dysphania ambrosioides* have been intensively used by the Cameroonian's traditional healers to treat epilepsy, depression, anxiety, psychoses and infantile convulsions [15]. The objective of this study was to evaluated the anticonvulsant effects of *Dysphania ambrosioides* in mice pilocarpine model of temporal lobe epilepsy to implication of antioxidant and GABAergic mechanisms.

2. Materials and methods

2.1. Plant collection and preparation of the aqueous extract

The aerial parts of *Dysphania ambrosioides* used in this experiment were harvested between March 2018 and April 2018, in Touboro, locality of the North Region of Cameroon. The area of study did not involve endangered or protected species. The collected species was identified at the National Herbarium of Yaoundé (Cameroon), where a voucher was deposited (85040/HNC). The aerial parts of *Dysphania ambrosioides* were crushed and the powder obtained (100 g) was macerated in 1000 mL of distilled water for 1 hour. The resulting mixture was boiled for 20 minutes and the supernatant was filtered using Whatman No. 1 filter paper. The resulting aqueous extract (decoction) was then administered orally to mice in a volume of 10 mL/kg. The decoction was concentrated using a rotary evaporator under reduced pressure at 50°C, and from this procedure, the extraction yield (8.7%) was calculated. After cooling, the mixture was filtered using Whatman number 1 paper (the volume of the residue is 23.51 mL), then the water was evaporated in an oven (at 60°C). From this process 0.87 g of dry extract of *Dysphania ambrosioides* were obtained. The concentration of the stock solution is 0.037 g/mL and calculated as follows: Concentration = (Mass of the dry extract)/ (volume of the decoction). The initial dose is 370 mg/kg. Three other doses were obtained after dilution of the stock solution at 1/2, 1/4, and 1/10 with distilled water. The solutions were administered to mice at a volume of 10 mL/kg of body weight and the doses used in this experiment were: 370, 185, 92.5, and 37 mg/kg.

2.2. Animal

Adult male Swiss mice weighting 20 - 25 g were obtained from the National Veterinary Laboratory, Garoua, Cameroon, and used throughout this experiment. They were housed in standard Plexiglas cages with food and water ad libitum. The animal house was maintained constantly at 25°C on a 12 h light-dark cycle. 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). In addition, the protocols for pharmacological studies were also realized in compliance with the recommendations provided in the Animal Research: Reporting of *In Vivo* Experiment (ARRIVE) guidelines [16].

2.3. Chemicals

Pilocarpine hydrochloride (PILO), Methyl-scopolamine, and all reagents used for the determination of oxidative stress markers and GABAergic transmission were from Sigma Chemical, USA. Sodium valproate (Depakine®) was from SANOFI, France.

2.4. Pilocarpine-induced temporal lobe epilepsy test

2.4.1. Induction of status epilepticus by pilocarpine

The first day of the test, seven lots of six mice received the following treatments: the normal and negative control received distilled water, the positive control received sodium valproate (300 mg/kg, intraperitoneally (i.p.)) and the aqueous extract of *Dysphania ambrosioides* (37, 92, 5, 185, 370 mg/kg, *per os* (*p.o.*)) for the test groups. Forty minutes after the different treatments, methyl-scopolamine (1 mg/kg, i.p) was administered to all groups except the normal control; twenty minutes later, *status epilepticus* was induced by the injection of a single dose of pilocarpine (400 mg/kg, i.p.), a cholinergic muscarinic receptor agonist [17, 18]. The mice were returned to their cages and the latency to *status epilepticus* onset was assessed. They were observed individually for 6 hours to determine the severity and duration of the epileptic seizures using the Racine scale [19]: stage 0: no response; stage 1: hyperactivity and clonus of the vibrissae; stage 2: head nodding, head clonus and myoclonic jerks; stage 3: unilateral clonus of the forelimbs; stage 4: elevation and bilateral clonus of the forelimbs; stage 5: tonic-clonic seizures with loss of righting reflex. Twenty-four hours after induction of *status epilepticus* and one hour after the different treatments, the following convulsion parameters were observed for a duration of 30 minutes per animal: the latency time of the first tonic-clonic seizure, and the time and the duration of tonic or clonic seizures. The latency times were expressed as a score as follows: Score = 1 – Latency of tonic-clonic seizures in the negative control group/Latency of tonic-clonic seizures in the other groups. The score was zero for all mice in the negative control and 1 for those which did not present any tonic-clonic seizure [19]. After administration of pilocarpine and observation of the animals in the previous test, they were treated daily for a week. the mice were sacrificed and the brain was removed to carry out measurements of the different biochemical parameters [18, 20].

2.4.2. Preparation of brain homogenates

One week after the daily treatment of mice, they were sacrificed by decapitation. Thereafter, brain of each mouse was quickly removed, cleaned with ice-cold saline (NaCl 0.9%) and stored at -80°C. The whole brain of each mouse was dissected out and divided into cortex and hippocampus. Subsequently the hippocampus of each animal was used to prepare homogenates [21, 22].

2.4.3. Determination of malondialdehyde level

Lipid peroxidation level in the frontal cortex and hippocampus homogenates was measured through the thiobarbituric assay [23]. The brain level of malondialhydehyde (MDA) expressed in µmol/g of tissue was calculated using Beer Lambert's formula and the extinction coefficient of 1.6 × 105 M/cm.

2.4.4. Quantification of reduced glutathione concentration

Glutathione was measured using the Ellman Method [24]. GSH was reacted with 5, 5′-dithio-bis-2-nitrobenzoic acid (Ellman's reagent) generating a yellow chromophore which was measured at 412 nm using a UV spectrophotometer and the concentration of glutathione was expressed in mol/g of tissue.

2.4.5. Measurement of nitric oxide level

Tissue nitric oxide level was measured in the brain using the Greiss reagent [25]. Briefly, 0.5 mL of Greiss reagent and 0.5 mL of tissue's supernatant were introduced into a test-tube and allowed to stand for 5 min, after which the absorbance of the mixture was read at 540 nm using a spectrophotometer. Nitric concentration expressed in mol/g of tissue sample was determined using sodium nitrate standard curve [21].

2.4.6. Catalase activity

The protocol described previously by Aebi was used to evaluate catalase activity in the tissue homogenate [26]. Briefly, to 125 µL of supernatant was added to 125 µL of 0.1 M phosphate buffer (pH 7.4) and 0.5 mL of 30 mM of hydrogen peroxide (H_2O_2). The absorbance was read at 240 nm for 30, 60 and 90 s using a spectrophotometer. Catalase activity was expressed as mmol of g of tissue.

2.4.7. Superoxide dismutase activity

The determination method of total superoxide dismutase activity in the tissue homogenate based on the inhibition of nitroblue tetrazolium reduction by the xanthine-xanthine oxidase system as a superoxide generator was used in this experiment [27]. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the nitroblue tetrazolium reduction rate.

2.4.8. Gamma-aminobutyric acid level

Ninhydrin reacts with succinic semialdehyde acid to form a complex, the absorbance at 512 nm wavelength of which is proportional to the amount of GABA. The amount of GABA in the homogenates was assessed by the colorimetric technique [63]. The amount of GABA in the homogenate was determined using GABA standards (50, 100, 150, 200, 250, 300, 350, and 400 µg), each mixed with 1.5 mg of glutamate dissolved in 2 mL of TCA (10%). The concentration of GABA was expressed in µg/g of tissue [21, 28].

2.4.9. GABA-transaminase activity

The coloration of the complex generated by succinic semialdehyde acid and 3-methyl-2-benzothia-zolone-2-hydrazone in the presence of 12% FeCl3 is proportional to the activity of GABA-transaminase (GABA-T) in the homogenate [29]. The amount of semialdehyde succinic acid generated during the incubation was quantified against the blank at 610 nm by a spectrophotometer at 30 and 90 s. The GABA-T activity was expressed in µg/min/mg of tissue [21].

2.5. Statistical analyses

Statistical analyses of the values obtained during our experiments were carried out using Graphpad Prism version 8.0.1 and Microsoft Office Excel 2016 software and XLSTAT. The results were expressed as mean ± standard error of the mean (ESM). The different values were compared using the analysis of variance (ANOVA) test. When differences existed, the "one-way ANOVA" multiple comparison test followed by the Tukey test was used to separate them. From $p<0.05$, the differences were considered significant.

3. Results

3.1. Effects of *Dysphania ambrosioides* aqueous extract on the protection against convulsions induced by pilocarpine

Animals treated with *Dysphania ambrosioides* extracts at the doses of 185 ($p<0.01$) and 370 mg/kg ($p<0.001$) were significantly protected against pilocarpine-induced tonic and clonic convulsions and mortality (Table 1).

Table 1 Effects of *Dysphania ambrosioides* aqueous extract on the percentage of protection against pilocarpine-induced convulsions

Groups	Numbers of mice	Numbers of deaths	Percentage of protection (%)
ED + ED	10	0	100
ED + PILO	10	5	50*
Da37 + PILO	10	3	70*
Da92.5 + PILO	10	3	70*
Da185 + PILO	10	2	80**
Da370 + PILO	10	1	90***
SV + PILO	10	1	90***

Each bar represents the mean ± SEM, n = 10 per group. Data were analysed by one-way ANOVA followed by Tukey's post-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$, significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; Pilocarpine, PILO; Sodium valproate SV.

3.2. Effects of *Dysphania ambrosioides* aqueous extract on the latency time to *status epilepticus* induced by pilocarpine

One-way ANOVA revealed a significant effect of *Dysphania ambrosioides* aqueous extract on the latency time to *status epilepticus* [F (6, 35) = 165.1; P < 0.0001] in the different groups. The latency time for the occurrence of the first tonic or clonic convulsion is 21.45 ± 2.99 (p<0.001) in the negative control, which is significantly greater than the latency time of animals in the normal control group (Figure 1). *Dysphania ambrosioides* delayed the onset time of convulsions by significantly increasing (p<0.001) this time in the groups treated with 185 and 370 mg/kg groups by 103.4% and 108.6%, respectively when compared with the negative control. Likewise, sodium valproate significantly increased this time by 115.9% (p<0.001) when compared with the negative control (Figure 1).

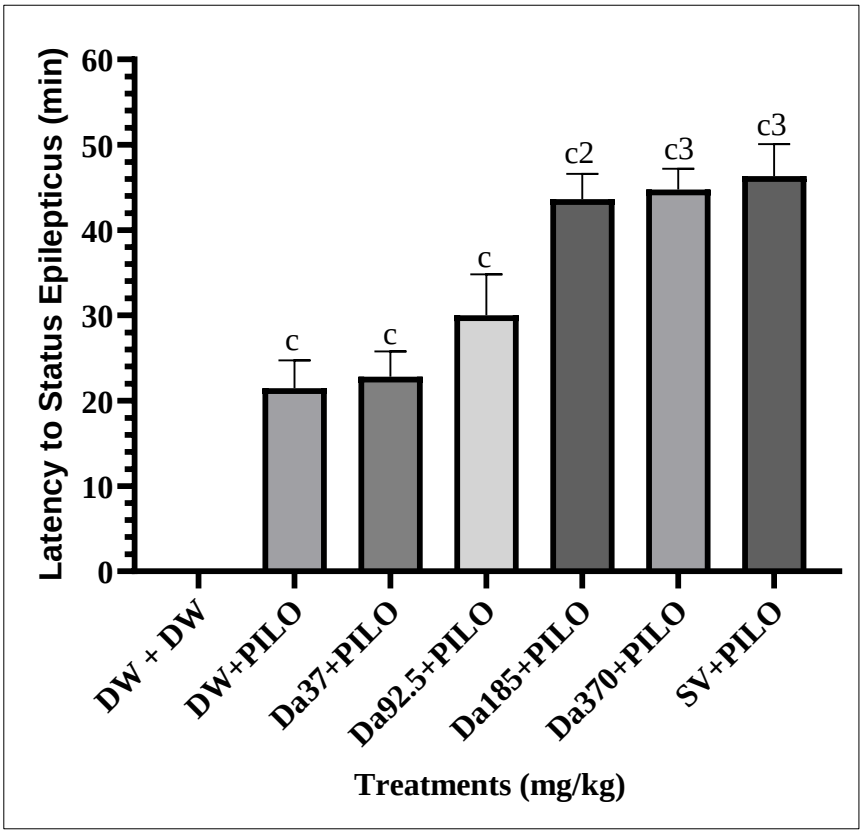

Figure 1 Effects of *Dysphania ambrosioides* aqueous extract on the latency time to *status epilepticus* induced by pilocarpine.

Each bar represents the mean ± SEM, n = 6. Data were analysed by one-way ANOVA followed by Tukey's post-test. [c]p<0.001 significantly different compared with normal control (DW + DW). [2]p<0.01, [3]p<0.001: significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; 360 mg/kg Pilocarpine, PILO; Sodium valproate SV.

3.3. Effects of *Dysphania ambrosioides* aqueous extract on the number and duration of clonic and tonic convulsions

One-way ANOVA revealed a significant effect of *Dysphania ambrosioides* aqueous extract on the number of clonic seizures [F (6, 35) = 198.5; P < 0.0001] in the different groups. *Dysphania ambrosioides* induced a significant reduction of the number of clonic seizures (p<0.001) at a dose of 370 mg/kg when compared to the negative control (Table 2). The same effect was observed in the positive control group treated with sodium valproate.

One-way ANOVA revealed a significant effect of *Dysphania ambrosioides* aqueous extract on the duration of clonic convulsions [F (6, 35) = 380.8; P < 0.0001] in the different groups. The duration of clonic convulsions significantly increased (p<0.001) in the negative control group when compared with the normal control group. The duration of clonic convulsions significantly decreases in a dose-dependent manner in the test groups treated with 37, 92.5, 185 and 370 mg/kg plant extract, respectively (Table 2).

One-way ANOVA revealed a significant effect of *Dysphania ambrosioides* aqueous extract on the number of tonic seizures [F (6, 35) = 131.1; P < 0.0001] in the different groups. The number of tonic seizures increased to 8.33 ± 0.81 (p<0.001) in the negative control compared to the normal control in whom no seizures were observed. *D. ambrosioides* induced a reduction of 32, 42 and 60% (p<0.001) at doses of 37, 92.5 and 370 mg/kg respectively compared to the negative control (Table 2).

One-way ANOVA revealed significant effect of *Dysphania ambrosioides* aqueous extract on the duration of tonic convulsions [F (6, 35) = 165.1; P < 0.0001] in the different groups. The duration of tonic convulsions decreases significantly in the test groups treated with the dose of 37, 92.5, 185 and 370 mg/kg, respectively when compared with the negative control group (Table 2). Sodium valproate used as an antiepileptic drug also induced a significant reduction of the number of tonic convulsions.

Table 2 Effects of *Dysphania ambrosioides* aqueous extract on the number and duration of clonic and tonic convulsions

Treatments	Doses (mg/kg)	Number of clonic convulsions	Duration of clonic convulsions (s)	Number of tonic convulsions	Duration of tonic convulsions (s)
ED + ED	- + -	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ED + PILO	- + 400	27.66 ± 3.93 ^{C}	56.33 ± 5.43 ^{c}	8.33 ± 0.81 ^{C}	23.23 ± 0.98 ^{C}
Da + PILO	37 + 400	23.83 ± 2.31 ^{C1}	30.33 ± 2.80 ^{c1}	5.66 ± 0.51 ^{C1}	20.50 ± 0.81 ^{C1}
Da + PILO	92.5 + 400	15.16 ± 1.16 ^{C2}	16.33 ± 1.50 ^{c3}	4.83 ± 0.75 ^{C2}	17.16 ± 0.51 ^{C1}
Da + PILO	185 + 400	6.16 ± 1.16 ^{C3}	7.67 ± 0.82 ^{c3}	5.16 ± 0.40 ^{C3}	15.00 ± 0.40 ^{C2}
Da + PILO	370 + 400	4.33 ± 1.21 ^{C3}	6.67 ± 1.03 ^{c3}	3.33 ± 0.51 ^{C3}	13.66 ± 0.75 ^{C3}
SV + PILO	300 + 400	2.16 ± 0.75 ^{C3}	6.17 ± 1.17 ^{c3}	2.50 ± 0.54 ^{C3}	12.66 ± 0.83 ^{C3}

Each value represents the mean ± SEM, n = 6 per group. Data were analysed by one-way ANOVA followed by Tukey's post-test. ^{c}p<0.001 significantly different compared with normal control (DW + DW); ^{1}p<0.05, ^{2}p<0.01, ^{3}p<0.001 significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; 360 mg/kg Pilocarpine, PILO; Sodium valproate

SV.4.1.4. Effects of *Dysphania ambrosioides* aqueous extract on oxidative stress markers and antioxidant enzymes

One-way ANOVA revealed significant inter-group differences in the SOD activity [F (7, 40) = 7.794; P < 0.0001]. Treatment with *Dysphania ambrosioides* extract increased the activity of SOD dose-dependently when compared with the negative control (Table 4).

One-way ANOVA revealed significant inter-group differences in the MDA concentration [F (7, 40) = 15.00; P < 0.0001]. The MDA concentration significantly decreased (p<0.001) in the test groups of mice when compared with the negative control (Table 4).

One-way ANOVA revealed significant inter-group differences in the GSH concentration [F (7, 40) = 211.5; P < 0.0001]. The aqueous extract of *Dysphania ambrosioides* significantly increased the concentration of GSH in a dose-dependent manner when compared with the negative control group (Table 4).

One-way ANOVA revealed significant inter-group differences in the NO concentration [F (7, 40) = 8.473; P < 0.0001]. *Dysphania ambrosioides* aqueous extract induced a significant decrease in the NO concentration when compared with the negative control group. Likewise, sodium valproate induced a significant reduction in the NO concentration (Table 4).

One-way ANOVA revealed significant inter-group differences in the CAT activity [F (7, 40) = 3.767; P < 0.0032]. Administration of *Dysphania ambrosioides* aqueous extract induced a significant increase of catalase activity in the test groups when compared with the negative control (Table 4).

Table 4 Effects of *Dysphania ambrosioides* aqueous extract on oxidative stress markers

Treatments		**ED +ED**	**ED + PILO**	**Da + PILO**	**Da + PILO**	**Da + PILO**	**Da + PILO**	**VS + PILO**
Doses (mg/kg)		- + -	- + 400	37 + 400	92.5 + 400	185 + 400	370 + 400	300 +400
SOD	(U/mg tissue)	28.56 ± 5.48	16.14 ± 2.30 ^{C}	30.53 ± 3.21 ^{3}	32.80 ± 2.62 ^{3}	34.82 ± 3.00 ^{3}	36.33 ± 0.91 ^{3}	37.79 ± 2.08 ^{a3}
MDA (μmol/g)		27.56 ± 5.29	39.65 ± 2.92 ^{C}	30.68 ± 3.04 ^{1}	26.56 ± 1.19 ^{3}	20.41 ± 0.87 ^{3}	20.56 ± 1.19 ^{3}	20.19 ± 0.56 ^{3}
CAT (U/mg tissue)		0,3 1.00 ± 0.10	0.20 ± 0.05 ^{b}	0.33 ± 0.03	0.42 ± 0.07 ^{2}	0.37 ± 0.04 ^{2}	0.36 ± 0.04 ^{2}	0.37 ± 0.05 ^{2}
GSH (μmol/mg tissue)		0.79 ± 0.05	0.37 ± 0.14 ^{c}	1.19 ± 0.13 ^{c2}	2.13 ± 0.,07 ^{b3}	3.34 ± 0.37 ^{a3}	3.43 ± 0.15 ^{a3}	3.41 ± 0.15 ^{a3}
NO	(U/mg tissue)	2.06 ± 0.35	4.02 ± 0.19 ^{b}	3.15 ± 0.86	3.06 ± 0.46	2.52 ± 0.15 ^{1}	2.17 ± 0.36 ^{1}	2.17 ± 0.53 ^{1}

Each value represents the mean ± SEM, n = 6 per group. Data were analysed by one-way ANOVA followed by Tukey's post-test. ^{c}p<0.001 significantly different compared with normal control (DW + DW); ^{1}p<0.05, ^{2}p<0.01, ^{3}p<0.001 significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; 360 mg/kg Pilocarpine, PILO; Sodium valproate SV; MDA: malondialdehyde, MDA; reduced glutathione, GSH; superoxide dismutase, SOD; catalase, CAT; nitric oxide, NO.

3.4. Effects of *Dysphania ambrosioides* aqueous extract on GABA concentration

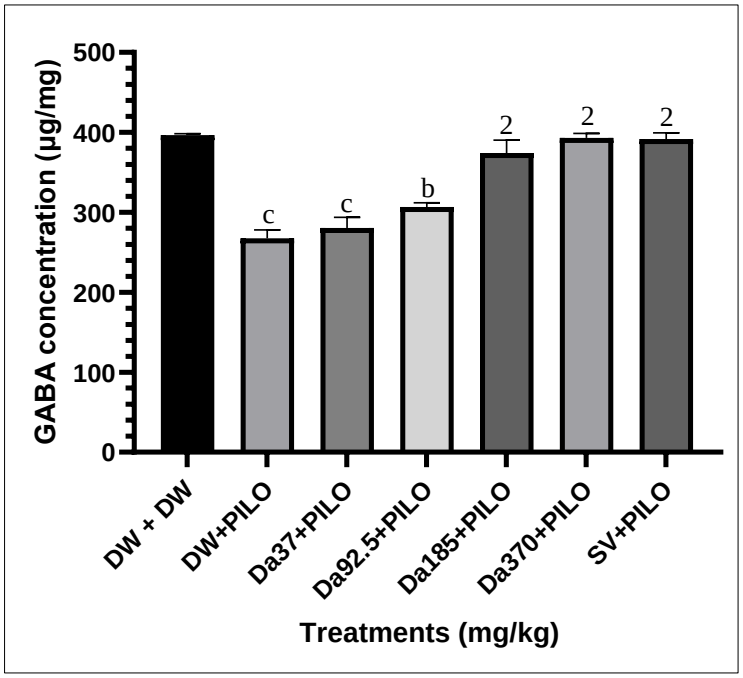

Figure 2 Effects of *Dysphania ambrosioides* aqueous extract on the concentration of GABA.

Each value represents the mean ± SEM, n = 6 per group. Data were analysed by one-way ANOVA followed by Tukey's post-test. ^{b}p<0.01, ^{c}p<0.001 significantly different compared with normal control (DW + DW); ^{2}p<0.01 significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; 360 mg/kg Pilocarpine, PILO; Sodium valproate SV.

One-way ANOVA revealed significant inter-group differences in the GABA level [F (7, 40) = 188.1; P < 0.0001]. The concentration of GABA decreased in the negative control when compared with the normal control. *Dysphania ambrosioides* aqueous extract induced a significant increase in the GABA level at the doses of 92.5, 185 and 370 mg/kg, respectively when compared with the negative control (Figure 2).

4.1.6. Effects of *Dysphania ambrosioides* aqueous extract on GABA-T concentration

One-way ANOVA revealed significant inter-group differences in the GABA-T activity [F (6, 35) = 209.8; P < 0.0001]. *Dysphania ambrosioides* aqueous extract induced a significant decrease in this activity of GABA-T in the test groups when compared with the negative control. The same effect was observed in the positive control group treated with sodium valproate. (Figure 3).

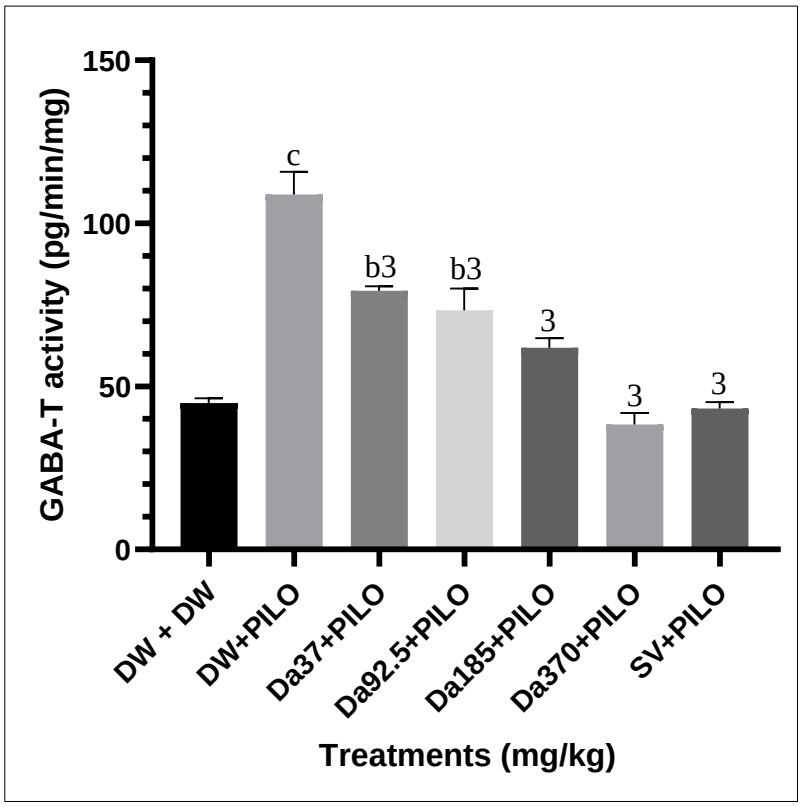

Figure 3 Effects of *Dysphania ambrosioides* aqueous extract on the activity of GABA-T.

Each value represents the mean ± SEM, n = 6 per group. Data were analysed by one-way ANOVA followed by Tukey's post-test. [b]p<0.01, [c]p<0.001 significantly different compared with normal control (DW + DW); [3]p<0.001 significantly different compared with negative control (DW +PILO). Distilled water, DW; 37 mg/kg *Dysphania ambrosioides* aqueous extract, Da37; 360 mg/kg Pilocarpine, PILO; Sodium valproate SV.

4. Discussion

The mice pilocarpine model is one of the well-established animal models of *status epilepticus* and shares many features of human MTLE [30]. Pilocarpine is a partial agonist of M1 muscarinic-type cholinergic receptors inducing limbic seizures in rodents with intraperitoneal or intracerebral injection [20]. Its binding to M1 receptors activates phospholipase C, producing diacylglycerol and inositol triphosphate. Inositol triphosphate allows the release of calcium contained in the endoplasmic reticulum; diacylglycerol activates protein kinase C which will allow the opening of membrane calcium channels. These mechanisms will increase the cellular concentration of calcium and therefore the excitability of the brain. This high concentration of calcium will cause the release of glutamate, inducing status epilepticus [31]. In this study, *Dysphania ambrosioides* aqueous extract increased the latency time for seizure onset, and decreased the number and duration of tonic and clonic seizures. The increase in the latency time for the onset of convulsions suggests that *Dysphania ambrosioides* has anticonvulsant properties [9, 22, 32] including anti-epileptogenic properties. These results could also be explained by the presence of terpenoids which, according to McPartland and Russo (2001), can cross the blood-brain barrier and increase the concentration of GABA. The hypothesis of a potentiation of GABA activity in the brain which reduces convulsions is probably accepted [33]. *Dysphania ambrosioides* would act like sodium valproate which is a broad-spectrum antiepileptic drug used for the treatment of generalized epilepsies and partial seizures [34].

Reactive oxygen species have been implicated in the development of pilocarpine-induced epileptic seizures and *status epilepticus* [35, 36]. The use of antioxidants for the treatment of epilepsy has a long history, with successful therapies

being described as early as the 1970s [37]. Numerous animal studies have demonstrated the use of certain antioxidants such as vitamin C, N-acetyl-cysteine, coenzyme Q10 and various plant extracts such as flavonoids for the treatment of SE, reduction of lipid peroxidation and restoration of the activities of different antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione levels in the hippocampus, striatum or cortex of rats [12, 38]. The results of this study showed that the decoction of *Dysphania ambrosioides* significantly increased the concentration of antioxidant enzymes such as SOD, catalase, reduced glutathione compared to the control and significantly decreased the concentration of MDA and NO compared to the negative control [22]. These results corroborate those of Taiwe *et al*. [39] who support the fact that, substances that antagonize the activity of lipid peroxidation markers in the cell have antioxidant properties which are due mainly to flavonoids, alkaloids and polyphenols which are very powerful antioxidant agents [40]. The results obtained indicate that the aqueous extract of *D. ambrosioides* has powerful antioxidant and anti-radical effects which could explain its anticonvulsant properties. A previous study of *Dysphania ambrosioides* shows that polyphenols in different extracts of the plant could contribute to the antioxidant activity of its samples and may be effective in the treatment of oxidative stress-mediated disorders [41].

The pathophysiology of the *status epilepticus* is not clearly understood, but excessive excitatory neurotransmission (glutamate) and loss of normal inhibitory neurotransmission (GABA) are considered the most likely mechanisms. The first-line therapies of choice are intravenous benzodiazepines (example: lorazepam and diazepam), which potentiate inhibitory responses mediated by GABA-A receptors [42]. Experiments on hippocampal cultures have shown that pilocarpine via muscarinic receptors causes an imbalance between excitation and inhibition which could result in seizures [43]. The significant decrease in the concentration of GABA in the negative control in the hippocampus of mice during the acute phase suggests a greater use of this amino acid in the attempt to counteract the hyperexcitability of the surrounding tissue [44]. In addition, GABA is metabolized into an inactive molecule, succinic acid semi-aldehyde, via an enzyme, GABA-T in neurons and glial cells [45] according to the equation: GABA (gamma-aminobutyrate) + 2-oxoglutarate gives succinate semialdehyde + L-glutamate [46]. The plant increased the concentration of GABA and decreased that of GABA-T in the groups treated with different doses of the aqueous extract of *Dysphania ambrosioides*. GABA-T decreases GABA levels in the brain and at the same time increases the levels of the excitatory neurotransmitter L-glutamate, thereby causing neurons to fire. The significant increase in GABA level and decrease in GABA-T in the hippocampus of mice treated with the aqueous extract of *Dysphania ambrosioides* in this study suggests a GABAergic mechanism of action of this plant. These properties could result from the presence in this aqueous extract of triterpenoids which have significant GABA-T inhibitory activity [47]. In this way, several antiepileptic drugs exert their action on the GABAergic system, which represents an important target for new antiepileptic drugs [48]. Indeed, the increase in the synthesis of GABA, its release, its affinity with allosteric receptors and the reduction of its *inactivation* are actions carried out by certain antiepileptics. The aqueous extract of *Dysphania ambrosioides* would act like sodium valproate which induces an increase in brain GABA by inhibition of semialdehyde succinyl dehydrogenase (GABA-degrading enzyme or GABA-T) or by activation of GABA synthesis by acid. glutamic acid decarboxylase [49]

5. Conclusion

In this work, the effects of *Dysphania ambrosioides* aqueous extract on pre- and post-*status epilepticus*, epileptic convulsions and oxidative/nitrosative stress in mice challenged with pilocarpine were shown. *Dysphania ambrosioides* aqueous extract significantly increased pre and post- *status epilepticus* latency and decreased the number and duration of clonic-tonic seizures. This protection would be on the one hand the consequence of the attenuation of certain dysfunctions due to crises such as oxidative stress and on the other hand the consequence of the regulation of the activity of GABA-T and eventually the raised level of GABA.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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, Yaoundé (No. FW-IRB00001954).

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