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# Anticonvulsant effect of *Asparagus africanus* Lam. root decoction on pilocarpine-induced temporal lobe epilepsy in white mice (*Mus musculus* Swiss)

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## Abstract

*Asparagus africanus* Lam. (Asparagaceae) is a widely used plant in traditional medicine as an anti-inflammatory, antioxidant, for the treatment of nervous disorders and epilepsy. The objective of this work was to study the anticonvulsant effects of *A. africanus* root decoction in white mice (*Mus musculus* Swiss) induced by pilocarpine. The experimental induction of "status epilepticus" and the evaluation of the anticonvulsant effects of *A. africanus* root decoction on pilocarpine-induced clonic and tonic convulsions were carried out. Seizure severity, latency, duration and number of clonics and tonics convulsions were evaluated. Concentrations of GABA, GABA-T, TNF-α and stress markers in the brains of mice were also estimated. *A. africanus* decreased the duration and number of clonic and tonic convulsions which increased the latency time of onset of clonic and tonic convulsions significantly and in a dose-dependent manner. GABA increased significantly in the brains of animals treated with *A. africanus* and a significant decrease of GABA-T and TNF-α. *A. africanus* also showed antioxidant effects. These results show that *A. africanus* has anticonvulsant effects. *A. africanus* would thus contain beneficial antiradical constituents in the treatment of epilepsy. These constituents would thus oppose free radicals. These results would justify the use of this plant in traditional medicine in the treatment of epilepsy.

**Keywords:** *Asparagus africanus*; Pilocarpine; Antioxidants; Anticonvulsants; Mouse.

## 1. Introduction

Epilepsy is a chronic disorder of the brain that results from excessive electrical discharges from neurons and is characterized by recurrent seizures manifested by brief episodes of involuntary shaking of part or all of the body [1]. Epilepsy is the most common neurological disorder after migraine, affecting all age groups [2]. It is the second leading cause of hospitalization in neurology after stroke [3]. Seizures in epilepsy can vary in intensity, ranging from brief loss of attention to severe and prolonged convulsions [4]. Epilepsy appears to the public as a shameful, dishonourable and supernatural disease [5]. It accounts for 0.6% of the global burden of disease and also has significant economic consequences [4]. Worldwide, about 70 million of people suffer from epilepsy, 80% are found in developing countries [6]. Epilepsy is diagnosed in 2.4 million people each year worldwide, the vast majority of whom are in low- and middle-income countries [4].

The causes of epilepsy can be: brain damage due to prenatal or perinatal trauma, congenital anomalies or genetic disorders associated with brain malformations, severe head trauma, stroke, infection affecting the brain and brain tumour etc [4].

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Different treatments have been developed to treat the symptoms [3]. On the other hand, despite the large number of molecules, the number of drug-resistant epilepsies does not seem to be decreasing [7]. A non-negligible percentage of patients (about 30%) continue to have seizures despite drug treatment. Temporal lobe epilepsies are among the most common forms of drug-resistant partial epilepsy [3]. They are drug resistant and surgery is one of the main therapeutic solutions [8]. The latter increases the risk of dead [3]. In addition, the lack of qualified personnel, the lack of health infrastructures and the high cost of surgery, shows that many patients do not have access to this practice [5].

Herbal pharmacotherapy for neurological diseases has been advancing due to fewer undesirable side effects and better tolerability [9]. Plants have extraordinary therapeutic virtues and it is estimated that nearly 75% of the African population has always used plants for self-care [10]. *A. africanus* also called "labbel fowru" (hyena spear) by the diamare fulbe in Cameroon, is used as a tuber by Musey and as an ingredient in porridge by kapsiki and fulbe in times of famine [11]. Aqueous and ethanolic extracts of the root of *A. africanus* were found to be less toxic and rich in phenolic compounds, a potential source of natural antioxidants that could be of great therapeutic importance in oxidative stress related to degenerative diseases [12]. *A. africanus* is widely used in traditional medicine as an anti-inflammatory [13], for the treatment of nervous disorders [14] and epilepsy. The objective of this work was therefore to evaluate the anticonvulsant effects of *A. africanus* root decoction on pilocarpine-induced temporal lobe epilepsy.

## 2. Materials

### 2.1. Plant material

The roots of *A. africanus* were collected in the town of Bini-Dang, in the Adamawa region (Cameroon). A sample of the plant has been deposited at the headquarters of the national herbarium in Yaoundé (Cameroon) under number 40168/HHC/Cam. The harvested roots of *A. africanus* were washed, dried at room temperature and then grounded. 500 mL of distilled water was added to 50 g of this powder and then brought to boil for 20 minutes on a hot plate set at 100 °C. After cooling, the solution was filtered using Wattman number 1 filter paper, then evaporated in an oven (70 °C) for 24 h. 325 mL of distilled water was added to the dry extract obtained constituting the mother solution, the dose of which is 254 mg/kg. Dilution with distilled water was made to 1/2 and 1/4 to obtain the 127 and 63.5 mg/kg doses respectively.

### 2.2. Animal material

White mice (*Mus musculus* Swiss) of both sexes, weighing 20 to 29 g were used for the various tests. These mice were obtained at the National Veterinary Laboratory (LANAVET) of Garoua (North Cameroon) and further raised in a controlled environment (12 hours of darkness), with access to unlimited food and water. All experiments were performed in accordance with the Guide to the Care and Use of Laboratory Animals published by "National Institutes of Health of the United States" (NIH Publication No. 85-23, revised in 1996). In addition, the studying protocol for the handling of animals and the procedure for the experiment were approved by the National Ethics Committee of Cameroon (Ref. No. FW-IRB00001954).

### 2.3. Drugs

Pilocarpine, methylscopolamine, Sodium valproate and all other chemicals and reagents used in the evaluation of the amount gamma-aminobutyric acid (GABA), gamma-aminobutyric acid transaminase (GABA-T) and stress marquers in the brain are from Sigma Chemical, USA. Kit type "Quantikine" (France) was used for the determination of TNF-α.

## 3. Methods

### 3.1. Experimental induction of "epilepticus status"

Mice homogeneously divided into six batches of five mice were treated with distilled water (10 mL/kg; *p.o*.) for the negative control batch, with different doses of *A. africanus* root decoction (63.5; 127 and 254 mg/kg; *p.o*.) for the test batches and with sodium valproate (300 mg/kg; i.p.) for the positive control batch. A neutral group of mice was treated with distilled water and did not receive pilocarpine or methylscopolamine. Thirty minutes later, the mice were then each treated by intraperitoneal injection of 1 mg/kg of methylscopolamine to reduce the peripheral effects of pilocarpine [15]. Clonic and tonic convulsions ("status epilepticus") were induced 30 minutes after administration of methylscopolamine to mice by intraperitoneal injection of pilocarpine at a dose of 360 mg/kg [16]. Injection of pilocarpine causes rodents to experience severe myoclonic tremors followed by multiple tonic and clonic convulsions and motor convulsions [16, 17]. The severity of convulsions was assessed in animals immediately after induction of

"epilepticus status" according to the Root Scale from 0 to 5 (level 0: no response; level 1: hyperactivity and vibrational clonus; level 2: nodding, head clonus and myoclonic shaking; level 3: unilateral forelimb clonus; level 4: rearing and bilateral forelimb clonus; level 5: tonic and clonic convulsions with loss of motor control reflex) for a duration of 6 hours [16, 17, 18]. Mice having reached level 5 severity were selected for the following experiments.

### 3.2. Anticonvulsant effects of *Asparagus africanus* root decoction on mice subjected to pilocarpine-induced "epilepticus status"

Anticonvulsant effects were evaluated in mice previously subjected to "epilepticus status" with spontaneous convulsions characteristic of temporal lobe epilepsy. 23 hours after injection of pilocarpine to the mice, the mice were again given distilled water (10 mL/kg) for the negative control lot, the different doses of *A. africanus* root decoction (63.5; 127 and 254 mg/kg) for the test lots and sodium valproate (300 mg/kg) for the positive control lot. One hour after the administration of the different treatments, several behavioral parameters were evaluated in each mouse for a period of 60 minutes. The parameters observed were the latency time of appearance of the first tonic and clonic convulsion, the duration and number of tonic and clonic convulsions [19]. After evaluation of these parameters, the mice received the various treatments mentioned above for seven days and were then sacrificed.

### 3.3. Gamma aminobutyric acid amounts

The amount of GABA in the hippocampus of mice was evaluated by the colorimetric technique of mouse brain homogenates described by Lowe [20]. The working reagent consisted of a mixture of 0.2 mL of 0.14 M ninhydrin solution prepared in a bicarbonate buffer solution (0.5 M; pH 9.9), and 0.1 mL of glacial trichloroacetic acid (TCA) 10%. A 100 µL homogenate sample was taken and introduced into the working reagent, the mixture was incubated at 60 °C in a water bath for 30 minutes. After cooling, the mixture was added into 5 mL of copper tartrate solution prepared from 0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid. The whole mixture was kept at a temperature of 25 °C for 10 minutes. The fluorescence resulting from the reaction between ninhydrin and GABA in the basic medium was measured using a spectrofluorimeter and was proportional to the concentration of GABA in the homogenates. A standard GABA solution was prepared parallelly from different GABA masses (50, 100, 150, 200, 250, 300, 350 and 400 µg) which were each mixed with 1.5 mg of glutamate dissolved in 0.1 mL of 10% TCA. The concentration of GABA in the samples was determined by referring to the GABA calibration curve [21]. The content of GABA in the brain was expressed in µg/g of brain tissue.

### 3.4. Determination of gamma aminobutyric acid transaminase

The activity of GABA-T was evaluated by the colorimetric assay method of Nayak and Chatterjee, [22]. 15 µmol of α-oxoglutarate, 15 µmol of GABA, 10 µg of pyridoxal phosphate, 0.1 mL of homogenate brain supernatant and 0.1 mL of 5% methanol were introduced in the tubes. The final volume of the mixture was made up to 3 mL with Tris-HCl buffer. The tubes were incubated at 37 °C for 30 minutes. The reaction was completed after adding 0.5 mL of 20% glacial TCA. Just before recording, the absorbance of each sample was recorded at 610 nm after 30 and 90s against a blank just after adding 1 mL iron chloride (12% $FeCl_3$). The color of the succinic semialdehyde acid and 3-methyl-2-benzothia-zolone-2-hydrazone complex formed in the presence of 12% $FeCl_3$ was proportional to the concentration of GABA-T in the homogenates. The activity of GABA-T was estimated in pg/min/g of tissue according to the Beer-Lambert law.

### 3.5. Evaluation of the antitumor necrosis factor-alpha (TNF-α)

The determination of TNF-α was performed by the enzyme-linked to immunosorbent assay (ELISA) method using "Quantikine" type kits (France). Primary antibodies specific for TNF-α were previously fixed at the bottom of the wells of a microplate. For this purpose, the primary antibody solution was introduced into the wells of a microplate and then the microplate was incubated for 24 h at 4°C in a water bath. The next day, the plate was washed 5 times with the wash buffer as provided by the kit, then 50 µL of the RD1-42 dilution solution was added to each well. Then, 50 µL of the specific protein standard was added to each well for the positive control wells, 50 µL of Tris buffer for the negative control wells, and 50 µL of homogenate for the test wells. The microplates were then incubated for 2 hours at 37°C, then each well was washed 5 times with the wash buffer. Subsequently, 100 µL of biotin-conjugated specific capture antibody was added to each well. A second incubation of the microplate was performed for 2 hours at 37°C followed by a series of 5 washes with Wash Buffer. Next, 100 µL of substrate, consisting of streptadivine coupled to peroxidase, was added to each well and the plate was incubated for 30 minutes at the temperature room and protected from light.

Finally, the enzyme reaction was stopped by adding 100 µL of stop solution ($H_2SO_4$) and then the absorbance for each well was read at 450 nm with a microplate ELISA reader. The concentrations of each cytokine were expressed in µg/mL, calculated by referring to the TNF calibration curve α, performed with the values provided by the kit.

### 3.6. Determination of Superoxide Dismutase (SOD)

The method used for this assay was that of Beauchamp and Fridovich [23]. An aliquot of 0.2 mL of sample was introduced into 2.5 mL of carbonate buffer (0.1M, pH 10.2). The reaction started with the addition of 0.3 mL of freshly prepared epinephrine to the mixture. After homogenization, the final mixture was read at 480 nm every 30 seconds up to 150 seconds to follow the increase in optic density. The reference vial contains 2.5 mL buffer, 0.3 mL adrenaline substrate and 0.2 mL distilled water. An absorbance unit of SOD activity will be defined as the amount of SOD required to cause 50% inhibition of the oxidation of adrenaline to adrenochrome for 1 min. The activity will be expressed in units per milligram of protein. The enzyme activity of SOD will be expressed as follows: *Inhibition Total x 100.*

### 3.7. Determination of malondialdehyde (MDA)

The degree of lipid peroxidation was achieved by a protocol previously described by Ohkawa et al., [24]. A volume of 100 µL of sample plus 500 µL of orthophosphoric acid (1%) sample and 500 µL of thiobarbituric acid/glacial acetic acid (ATB 1% / AAcl 1%) were combined to form a mixture which was placed in a boiling water bath for 15 min at 100°C. The tubes were then cooled in an ice bath. The absorbance of the supernatants was read at 530 nm against the white. The concentration of MDA is expressed in nanomoles per milligram of protein (nmol/mg prot) according to the following formula:

$DO = \varepsilon.L.\ C;\ C = DO/\ (\varepsilon.L)$

### 3.8. Catalase activity (CAT)

CAT activity was determined in tissues according to the method described by Nassima et al., [25]. A substrate solution consisting of 1 mL of phosphate buffer (0.1M, PH 7.4), 950 µL of hydrogen peroxide ($H_2O_2$) solution (0.019) and 250 µL of the enzyme source was formed. The measurement of CAT activity was followed by recording the absorbance at 570 nm of the reaction mixture every minute for two minutes. The enzymatic activity of CAT was deduced by the Beer-Lambert law: $CAT\ activity = \varepsilon.\ L.\ C$

### 3.9. Determination of reduced glutathione (RG)

The determination of RG was carried out according to the protocol described by Ellman [26]. To perform this assay, 1.5 mL of Ellman's reagent was introduced into test tubes previously containing 100 µL of homogenate and 100 µL of phosphate-saline buffer (0.1M, pH 7.4). The tubes were shaken and left to stand for incubation for one hour at room temperature, then the absorbance was read on the spectrophotometer at 412 nm against the blank. The amount of RG was determined using the following formula:

$$[Reduced\ glutathione]\ (mol/g\ tissue) = \left(\frac{DO \times Vt}{\varepsilon \times L \times Vi \times m}\right) \times 2$$

### 3.10. Statistical analysis

The statistical analysis was carried out using GraphPad Prism software version 8.0.1. The results were expressed as mean ± standard error (S.E.M). The different values were compared using analysis of variance (ANOVA) and Tukey's multiple comparison.

## 4. Results

### 4.1. Effects of *Asparagus africanus* root decoction on the severity of tonic and clonic convulsions induced by pilocarpine

Pilocarpine (360 mg/kg) injected intraperitoneally one hour after administration of the negative control to mice with distilled water caused tonic and clonic convulsions which are characteristic of the epileptic condition also called "status epilepticus". The different doses of *A. africanus* root decoction (63.5, 127 and 254 mg/kg) did not protect the mice against pilocarpine-induced status epilepticus. Sodium valproate (300 mg/kg) injected intraperitoneally, which is an

antiepileptic reference, also did not show a significant effect against "status epilepticus" in mice. In this experiment, all mice reached the highest severity of status epilepticus, i.e. level 5 on the Root Scale.

### 4.2. Effects of *Asparagus africanus* root decoction on the latency time, duration and number of tonic and clonic convulsions induced by pilocarpine

All doses *A. africanus* roots decoction increased the latency time of tonic and clonic convulsions [(F (5, 24) =477.8; P <0.001); $R^2$ = 99.01%] (Figure 1), decreased the duration of tonic and clonic convulsions [(F (5, 24) =218.90; P <0. 001); $R^2$ = 97.85%] (figure 2) and reduced the number of tonic and clonic convulsions [(F (5, 24) =85.24; P <0.001); $R^2$ = 94.67%] (figure 3); significantly comparable to sodium valproate which is a antiepileptic reference as compared to the negative control lot.

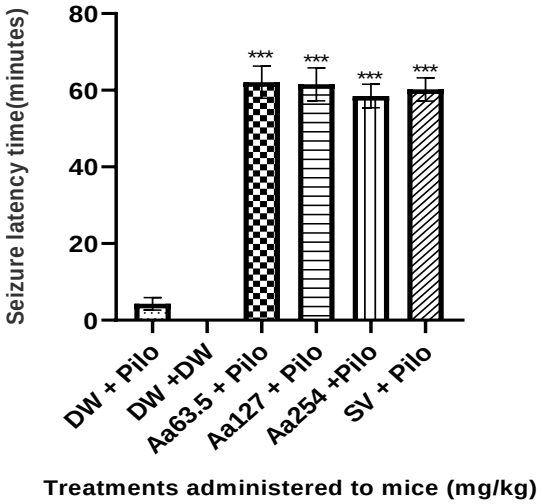


**Figure 1:** Effects of *Asparagus africanus* root decoction on the latency time of pilocarpine-induced tonic and clonic convulsions.

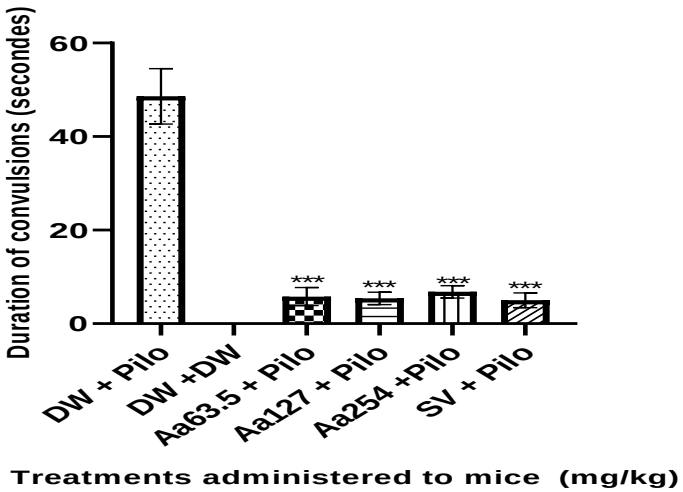


**Figure 2:** Effects of *Asparagus africanus* root decoction on the duration of pilocarpine-induced tonic and clonic convulsions.

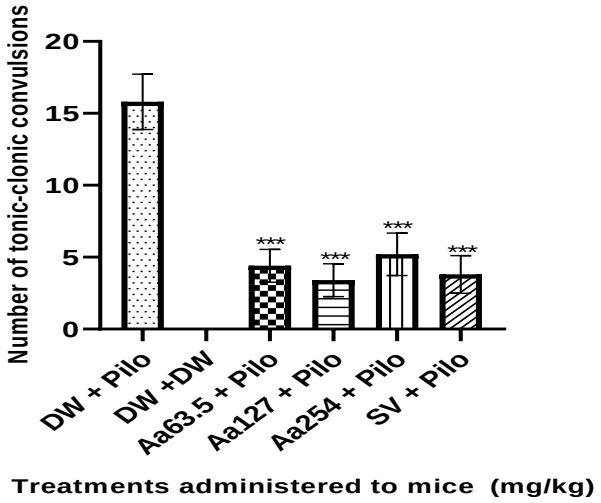


**Figure 3:** Effects of *Asparagus africanus* root decoction on the number of tonic and clonic convulsions induced by pilocarpine.

Each bar represents the mean ± ESM, n = 5. ***p ≤ 0.001, significant difference from negative control. DW + Pilo: negative control consisting of pilocarpinized mice treated with distilled water, DW + DW: normal control consisting of mice treated with distilled water, SV: positive control consisting of mice treated with sodium valproate (300 mg/kg), *Aa: Asparagus africanus*.

### 4.3. Effect of *Asparagus africanus* root decoction on gamma-aminobutyric acid, gamma-aminobutyric acid transaminase and antitumor necrosis factor-alpha in the hyppocampus of mice epilepticised by pilocarpine

Table 1 shows a significant depedent doses increase in GABA concentration [(F (5, 24) = 134.7; P < 0.001); $R^2$ = 99.12%], decrease in GABA-T concentration [(F (5, 24) = 29.54; P < 0. 001); $R^2$ = 96.10%] and decrease in TNF-α concentration [(F (5, 24) = 87.45; P<0.001); $R^2$ = 98.65%] comparable to normal mice and mice treated with sodium valproate (positive control) compared to the negative control lot.

**Table 1:** Effect of *Asparagus africanus* root decoction on **gamma-aminobutyric acid, gamma-aminobutyric acid transaminase and antitumor necrosis factor-alpha**.

| Treatments | Doses (mg/kg) | GABA (µg/g) | GABA-T (µg/g) | TNF-α |
|---|---|---|---|---|
| DW + DW | - + - | 374.54 ± 4.68*** | 33.77 ± 4.38*** | 291.99 ± 5.24*** |
| DW + Pilo | - + 360 | 293.98 ± 5.07$_c$ | 77.56 ± 6.45$_c$ | 371.39 ± 6.90$_c$ |
| Aa + Pilo | 63.5 + 360 | 385.15 ± 3.88*** | 30.98 ± 0.11*** | 289.72 ± 3.99*** |
| Aa + Pilo | 127 + 360 | 384.83 ± 4.41*** | 41.27 ± 2.75** | 334.69 ± 4.59*$_b$ |
| Aa +Pilo | 254 + 360 | 376.20 ± 3.07*** | 48.97 ± 6.46** | 356.32 ± 5.16$_c$ |
| SV +Pilo | 300 + 360 | 369.99 ± 4.08*** | 36.50 ± 3.24*** | 319.04 ± 3.75**$_a$ |

Each value represents the mean ± ESM; n = 5. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001: significant difference from negative control; $^a$p ≤ 0.05, $^b$p ≤ 0.01, $^c$p ≤ 0.001: significant difference from normal control; DW + Pilo: negative control consisting of pilocarpinized mice treated with distilled water; DW + DW: normal control consisting of mice treated with distilled water; SV: positive control consisting of mice treated with sodium valproate (300 mg/kg); *Aa: Asparagus africanus*.

### 4.4. Effects of *Asparagus africanus* root decoction on malondialdehyde, catalase, reduced glutathione and superoxide dismutase in the hyppocampus of mice epilepticised by pilocarpine

Table 2 shows that administration of *A. africanus* root decoction resulted in a significant decrease in MDA [(F (5, 24) = 34.45; P<0.001); $R^2$ = 93.49%] and a significant increase in CAT [(F (5, 24) = 126.60; P<0. 001); $R^2$ = 98.14%], RG [(F (5, 24) = 135; P<0.001); $R^2$ = 99.12%] and SOD [(F (5, 24) = 55.15; P<0.001); $R^2$ = 97.87%] in epileptic mice comparable to mice treated with sodium valproate (positive control) compared to the negative control lot.

**Table 2:** Effect of *Asparagus africanus* root decoction on malondialdehyde, catalase, superoxide dismutase and reduced glutathione in the hippocampus of pilocarpine-treated mice with epilepsy.

| Treatments | Doses (mg/kg) | MDA (nmol/mg) | CAT (mM/min/mL) | SOD (U/mL) | RG (mol/g) |
|---|---|---|---|---|---|
| DW + DW | - + - | 1.02 ± 0.01*** | 404.76 ± 1.39*** | 22.15 ± 0.01*** | 315.44 ± 1.04*** |
| DW + Pilo | - + 360 | 1.29 ± 0.03$_c$ | 352.64 ± 5.55$_c$ | 12.45 ± 0.00$_c$ | 254.41 ± 4.16$_c$ |
| Aa + Pilo | 63.5 + 360 | 1.03 ± 0.02*** | 372.68 ± 2.40$_c$*** | 17.95 ± 0.00$_b$*** | 295.22 ± 2.60$_b$*** |
| Aa + Pilo | 127 + 360 | 1.15 ± 0.02$_b$** | 363.86 ± 1.39$_c$** | 16.65 ± 0.01$_c$** | 281.62 ± 3.12$_c$*** |
| Aa + Pilo | 254 + 360 | 1.21 ± 0.06$_c$ | 357.45 ± 3.67$_c$ | 15.80 ± 0.00$_c$* | 276.47 ± 2.08$_c$** |
| SV + Pilo | 300 + 360 | 1.02 ± 0.00*** | 391.91 ± 2.40$_b$*** | 18.80 ± 0.00$_a$*** | 303.31 ± 1.56$_a$*** |

Each value represents the mean ± ESM; n = 5. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001: significant difference from negative control; $^a$p ≤ 0.05, $^b$p ≤ 0.01, $^c$p ≤ 0.001: significant difference from normal control; DW + Pilo: negative control consisting of pilocarpinized mice treated with distilled water; DW + DW: normal control consisting of mice treated with distilled water; SV: positive control consisting of mice treated with sodium valproate (300 mg/kg); Aa: *Asparagus africanus*.

## 5. Discussion

The administration of *A. africanus* root decoction protected the mice against pilocarpine-induced convulsion severity (360 mg/kg) by significantly increasing the latency time of tonic and clonic convulsions on the one hand, and by decreasing the duration and number of tonic and clonic convulsions on the other hand. Pilocarpine is a cholinergic agonist that binds to acetylcholine (M1) at muscarinic receptors. At the cerebral level, these receptors are expressed in the cortex, hippocampus, amygdala, basal ganglia and striatum [27]. Activation of M1 receptors leads to the genesis of epileptic seizures via secondary release of glutamate [28]. The delay in the onset of pilocarpine-induced convulsions suggests that decoction of *A. africanus* roots has anticonvulsant properties. It antagonizes pilocarpine-induced seizures, suggesting an interaction with GABAergic neurotransmission [29, 30, 31]. In the present study, pilocarpine induced tonic and clonic convulsions in mice from the negative control batch. Protection against pilocarpine-induced convulsions suggests that decoction of *A. africanus* would have anticonvulsant properties and could be used against partial epilepsy, specifically temporal lobe epilepsy [32]. Sodium valproate has anticonvulsant effects with a broad spectrum of activity. The protective effect of the decoction of *A. africanus* roots and sodium valproate on pilocarpine-induced convulsions is believed to result in the neural mechanism of inhibition of voltage-dependent sodium channels in favour of increasing GABA concentration at central nervous system synapses with facilitation of GABAergic neurotransmission, reduction of N-methyl-D-aspartate and glutamate receptors, and with attenuation of nerve inflammation [33]. These observations suggest that decoction of *A. africanus* roots could have altered the epileptogenesis process by increasing GABAergic activity and reducing excitatory neurotransmission [34].

In the GABAergic neurotransmission studies of *A. africanus* root decoction in pilocarpine-induced temporal lobe epilepsy, the concentration of GABA and the activity of GABA-T were evaluated. GABA is the major inhibitory neurotransmitter in the brain and is widely implicated in epilepsy. GABA-ergic neurotransmission or activity is known to inhibit or attenuate seizures [35]. Decoction of *A. africanus* roots significantly and dose-dependently increased GABA concentration and decreased GABAtransaminase activity. Any substance that decreases GABAtransaminase activity by increasing GABA concentration would have antiepileptic effects [36]. GABA is thus involved in the mechanisms of action of anticonvulsants [37]. The cerebral increase of GABA would be due to the stimulation of the activity of GABAergic neurons by the decoction of the roots of *A. africanus* which would release more GABA. This is the case of sodium valproate, an antiepileptic that induces an increase in the amount of cerebral GABA by inhibiting GABA-T (GABA-T-degrading enzyme) or by activating GABA synthesis with Glutamic acid decarboxylase [38, 39]. The increase in GABAergic neurotransmission would therefore be responsible for the anticonvulsant properties. Given that the decoction of *A. africanus* roots induced the same effects and often better than sodium valproate, a standard anti-epileptic, the decoction of *A. africanus* would thus have anti-epileptic properties.

According to the work of Auvin and Sankar, [40], there is a reciprocal relationship between epileptic seizures and cytokines. On the one hand, pro-inflammatory cytokines have been shown to have pro-convulsant properties. On the other hand, a state of epilepsy is responsible for the cerebral expression of inflammatory cytokines such as IL- 1β, IL-6 and TNF-α. TNF-α is a ubiquitous inflammatory cytokine that acts via membrane receptors on the NF-κB signalling pathway. To evaluate the anti-inflammatory effects of *A. africanus* root decoction, TNF-α was assayed in pilocarpine-induced mouse hypocammal. TNF-α is elevated in the brains of patients [41]. According to Mogi and collaborators [42], excess TNF-α is toxic to cells although at a low dose it has neurotrophic effects on hippocampal neurons and protects neurons in culture from glutamate, free radicals, and the toxicity of β-A [43]. Decoction of the roots of *A. africanus* decreased the concentration of TNF-α significantly and dependently the dose. These results are in concord with the work of Hassan and collaborators [13] who show that *A. africanus* has anti-inflammatory effects.

Determination of the antioxidant effects of *A. africanus* root decoction was done by evaluating the levels of MDA, RG, SOD and CAT in the hipocammus of pilocarpine-induced mice. The results obtained show that the decoction of *A. africanus* roots significantly and dose-dependently increased the concentration of RG, SOD and CAT. SOD eliminates the superoxide anion by a dismutation reaction, transforming it into hydrogen peroxide and oxygen. Glutathione peroxidase reduces peroxides by removing lipid peroxides resulting from the action of oxidative stress on polyunsaturated fatty acids [44] and CAT removes $H_2O_2$ and its toxic radicals resulting from the antioxidant action of SOD [34]. These results suggest that the decoction of *A. africanus* roots would have antioxidant effects. Most of these antioxidants produce dioxides and peroxyl radicals, the latte, after being transform into a cyclic peroxide, release various toxic aldehydes including MDA [45]. The decoction of the roots of *A. africanus* significantly decreased the concentration of MDA. Furthermore, these effects are comparable to those of sodium valproate, a drug known to prevent oxidative stress [46, 47]. These results corroborate with the work of Onyeka, [12] who shows that *A. africanus* has good antioxidant potential. The decoction of *A. africanus* roots would thus have beneficial antiradical constituents in the treatment of epilepsy, since these constituents oppose free radicals when the body's antiradical defense system is deficient.

## 6. Conclusion

The objective of this work was to demonstrate the anticonvulsant effects of the decoction of the roots of *A. africanus*. The decoction of the roots of *A. africanus* has anticonvulsant properties that were visible in the antagonism of pilocarpine-induced epileptic seizures. It also showed anti-inflammatory effects by decreasing the concentration of TNFα which at high concentrations is toxic to neurons. The decoction of the roots of *A. africanus* has also shown antioxidant effects. *A. africanus* would thus contain beneficial antiradical constituents in the treatment of epilepsy. The antiepileptic activity would thus be due to their actions on the radicals that destabilize the neuronal membranes. These results would justify the use of this plant in traditional medicine as an anticonvulsant, antioxidant and anti-inflammatory.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

All authors declare that they have no conflict of interest.

### *Statement of ethical approval*

The experiment was carried out at the Laboratory of Medicinal plants, Health and Galenic Fomulation, Faculty of Sciences, University of Ngaoundéré in accordance with approval by the National Ethics Committee of Cameroon (Ref. No. FW-IRB00001954).

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