Preventive effects of *Ocimum gratissimum* (Lamiaceae) leaf decoction on pilocarpine-induced seizure and anxiety in white mice *Mus musculus* Swiss

Abstract

The objective of this work was to evaluate the effects of leaf decoct of *Ocimum gratissimum* (24.4, 61, 122 and 244 mg/kg) on seizures and anxiety during pilocarpine-induced epileptogenesis in mice. To evaluate anticonvulsivant and anxiolytic effect of *O. gratissimum*, a pathological model of pilocarpine-induced temporal epilepsy, behavioral tests and analyses of biochemical parameters (malondialdehyde, reduced glutathione, GABA and GABA-transaminase) were used to verify its properties on pilocarpine-induced seizures, oxidative stress and anxiety. Four different doses of the decoction of *O. gratissimum* were administered to the animals, and their effects were compared with distilled water (10 mL/kg), pilocarpine (360 mg/kg), sodium valproate (300 mg/kg), NaCl 0.9% and diazepam (3 and 0.3 mg/kg). The results showed that administration of *O. gratissimum* significantly decreases the number and duration of seizures, it increases the latency of status epilepticus and seizures, as well as the seizure score. The decoctate significantly improves the behavioral performance of mice, and inhibits the effects of pilocarpine. This resulted in a decrease in MDA levels, an increase in reduced glutathione levels. A significant increase in GABA levels and a decrease in GABA-transaminase activity were also observed in animals treated with 244 mg/kg. These results suggest that *O. gratissimum* has anticonvulsant, antioxidant and anxiolytic properties. It could be used as a preventive measure against Temporal Lobe Epilepsy.

Keywords: *Ocimum gratissimum*, anticonvulsant, anxiolytic, pilocarpine, status epilepticus.

1. Introduction

The nervous system is the control centre for all body activities [1]. He is subject to many disorders, including epilepsy [1]. Epilepsy is a chronic disorder of the normal electrical activity of the brain, characterized by recurrent unprovoked seizures [2]. Patients with epilepsy often have comorbidities including psychiatric disorders, particularly depressive disorders and anxiety disorders [3]; with prevalences of up to 50% in drug-resistant forms [4]. Patients suffering from anxiety as a comorbidity of epilepsy describe it as more deleterious than the seizures themselves [3], especially since their management often remains complex [5].

However, the pharmacological treatment of epilepsy and its complications is based on the use of antiepileptics, these act by a direct effect on the neural mechanisms triggering the

seizures; have only a suspensive effect on the latter, and do not cure the underlying pathological processes of epileptogenesis, causing their recurrence and progression [6,7]. Associated with disease resistance, their undesirable side effects and toxicity, their use therefore has limits [8, 9,10]. Based on this observation, the use of antiepileptic medicinal plants can then be perceived as an alternative to conventional drugs, and a hope for indigent populations [11, 12].

Ocimum gratissimum is a plant used very actively in the traditional system all over the world. Its stems are used in infusion for the treatment of epilepsy in children [13]. In Cameroon and northern Brazil, it is used as a condiment for culinary, ornamental, ritualic purposes [14, 15]. The people of Benin use it to treat diarrhea, dysentery, hypertension, candidiasis, diabetes in pregnant women and Buruli ulcer [16, 17, 18], abdominal pain, eye inflammation, ear infections, fever, cough, infertility, regulation of menstruation [19]; among other uses. In addition, no scientific evidence of the pharmacological properties of its decoct on epileptogenesis has been reported to date. The present study was conducted to test the hypothesis that the decoction of *O. gratissimum.* has anticonvulsant and anxiolytic properties due to its use in traditional medicine in Cameroon to treat epilepsy.

2. MATERIAL AND METHOD

2.1. Material

2.1.1. Plant biological material and preparation of extracts

The leaves of *Ocimum gratissimum* were harvested in the morning in the city of Yaoundé (Adamaoua-Cameroon), and were dried at room temperature of 25 ° C for a week. A sample of the plant was deposited at the National Herbarium of Yaoundé and identified under the number 73616HNC. The leaves were crushed with a mortar and sieved to obtain a powder. A decoction was prepared by introducing 10g of dry powder of *O. gratissimum* into a tube containing 50 ml of distilled water. The set was brought to a boil for 20 minutes on a hot plate. After cooling, the mixture was filtered using Whatman paper number 1, then the water was evaporated in the oven (at 60 ° C). The process made it possible to obtain 0.83 grams of dry extract of *O. gratissimum*, a yield of 8.3%. At the end of the preparation of this decoction, we obtained a stock solution of concentration 24.4 mg/mL. Three other solutions of different concentrations were obtained by diluting the stock solution to 1/2, 1/4 and 1/10th with distilled water. As the solutions were administered to mice at a dose of 10 mL/kg body weight, the doses studied in our experiments were as follows: 244; 122; 61 and 24.4 mg/kg.

2.1.2. Phytochemical analysis of *Ocimum gratissimum*

Chemicals tests were carried out on the extract using standard procedure to identify the constituents as described by Pessoa et al., (2002) ; Soforwa (1984) ; Trease and Evans (1984) [20, 21, 22].

2.1.3. Animal biological material and treatments

Male and female white mice belonging to the *Mus musculus* Swiss strain, weighing between 18 and 31g, were used to carry out the various tests of this study. They were obtained from the animal the National Veterinary Laboratory of Garoua, Cameroon. They were housed in standard Plexiglas cages, maintained constantly at 25 C on a 12-hour/12-hour light/dark cycle in the laboratory of science of the University of Ngaoundere (Cameroon), where experiments were carried out. The animals were acclimatized 72 hours before the start of the experiments and their diet consisted of water ad Libitum and balls of corn bran mixed with wood cake. The mice were weighed and divided into 8 groups as shown in Figure 1. After administration of the various treatments to the animals, tonic and clonic convulsions, characteristic of the initial state of status called status epilepticus (SE), were induced in mice by intraperitoneal injection of pilocarpine. The injection protocol was similar to those previously described by Curia *et al.,* and Magnin [23, 24]. A single low dose of N-methyl-scopolamine (1 mg/kg, *i.p.*) was previously administered to the animals 40 minutes after administration of the initial treatments (distilled water, doses of *O. gratissimum*, Diaz) to reduce the cholinergic effects of pilocarpine in the periphery (diarrhea, piloerection, chewing, lacrimation, tremors, spasms etc.). 30 minutes after this injection, the animals received a single injection of pilocarpine (360 mg/kg, *i.p.*), then they were returned to their cages and observed individually to determine the latency time before the onset of SE. Mice that did not convulse and died were said to be protected against tonic-clonic seizures [25]. 24 hours after pilocarpine injection, they were observed again over a period of 30 minutes to determine the latency time of the first attack, the duration and number of seizures, and characterize them according to tonic or clonic type. Subsequently, these mice were subjected to the various tests (Open Field (OF) and raised cross labyrinth (EPM) for a period of 5 minutes, after which they were sacrificed, and their hippocampus was taken for biochemical parameters (reduced gluthation, malondialdehyde, GABA and GABA-T).

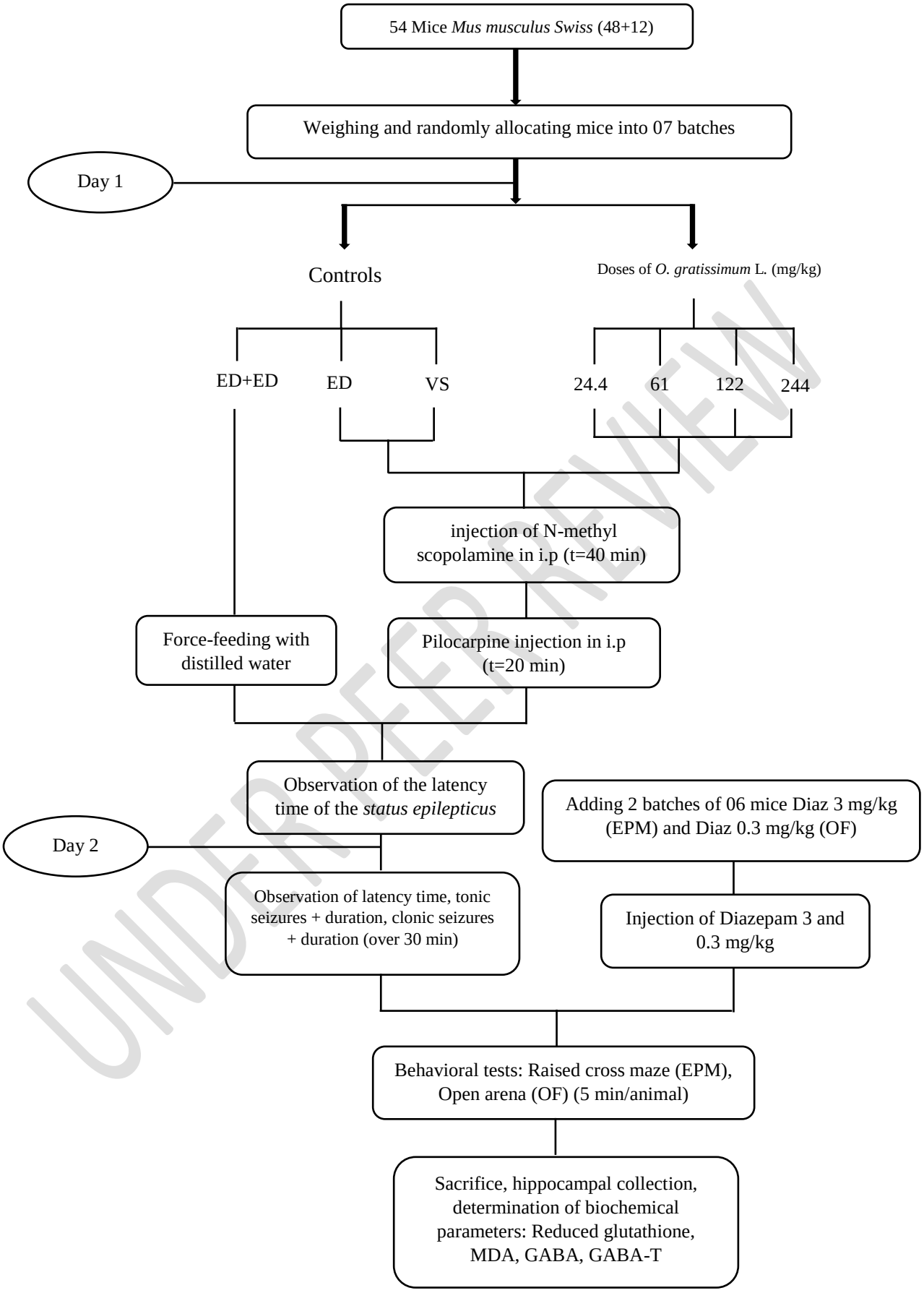

Figure 1: Summary diagram of the experimental protocol

Legend: (42+12): Of the 54 starting mice, only 42 were treated on the first day of manipulation; the last twelve were used on the second day to serve as additional control lots, for behavioral tests (Diaz). ED+ED= Normal control: control batch receiving only 0.9% NaCL; ED= negative control: control batch initially received distilled water; VS= Positive Control: mice given Sodium Valproate; diaz = positive control; mice given Diazepam 3 mg/kg (EPM), Diazepam 0.3 mg/kg (OF). 24.4, 61, 122, 244 = mouse having received the decoction of *O. gratissimum* 'i.p' denotes the administration of the substance intraperitoneally. MDA = malondialdehyde; GABA=Gamma aminobutyric acid; GABA-T= GABA-transaminase.

2.2. Method

The animal's experiments were carried out in accordance with the guidelines of the Bioethics Commettee of Cameroon (No. FWA-IRB00001954) and international principles of laboratory animal care (NIH Publication 8023, revised 1996). Every effort has been made to minimize animal suffering and reduce the number of animals used in experiments.

2.2.1. Pharmacological tests

Behavioural tests were used to assess the anxiolytic effect of *O. gratissimum*

2.2.1.1. Test of the raised cross labyrinth

This test based on the study of the spontaneous behavior of the animal was carried out according to the protocol described by Ngo Bum et al. [26]. The mice given the decoction of *O. gratissimum* and pilocarpine were used in this study. A group of five normal animals given diazepam 0.3 mg/ml (Diaz) was formed, serving as a positive control for the anxiety test. The test consisted of placing the mice one after the other in the center of the device, facing an open arm, so as to allow them to explore freely. The number of entries and time spent in each type of arm, the number of 'rearing' (when the animal stands on its hind legs and leans on the edges of the experimental set-up) and 'head dipping' (when the animal stands in an open arm of the maze and looks below the experimental set-up) were noted for a period of 5 minutes for each animal. An entry into an arm type was defined as the moment when the animal placed its four legs within the inner limits of the arm. After each observation, the mouse was removed from the device and returned to its cage, then the device was cleaned with ethyl alcohol (70 °C). The number of entries and the time spent in each arm type were expressed as averages.

2.2.1.2. Open field test

This test, commonly used to assess the level of locomotor activity, level of exploration and emotional reactivity in rodents, was performed according to the protocol described by Ngo

Bum et al. [26]. After passing through the maze, the mice were put back in their starting cages for an hour to reduce neophobic responses related to the experimental environment. Another group of five normal animals (Diaz) treated with diazepam (0.3 mg/ml, *i.p.*) was formed and added to the other groups, serving as a positive control. The test consisted of placing the mice one after the other in the center of the device, so as to allow them a free exploration. The number of crossings, the number of groomings (when the animal cleans itself in the arena), the frequency of rearing (when the animal stands on its hind legs and leans on the edges of the experimental set-up), and the time spent in the center were recorded for a period of 5 minutes for each animal. After each observation, the mouse was returned to its cage and then the device was cleaned with ethyl alcohol (70 °C).

2.2.2. Biochemical assays

After the open arena test, all animals were sacrificed by decapitation, brains were removed, washed in 0.9% NaCl, wrung out on toilet paper, weighed and placed in boxes containing saline (NaCl 0.09%), frozen for solidification for one hour. After solidification, the organs were dissected on a cold-held dissection table to extract the hippocampus. For each animal, the mass of the hippocampus thus collected was evaluated, then in a sample of this hippocampus (0.1 g) was added 1 mL of Tris buffer (HCl 50 mM; KCl 150 mM; pH 7.4). After grinding, the mixture was fed into a marked tube and then centrifuged at 10,000 revolutions per minute for 15 minutes. The supernatant was pipetted and fed into an eppendorf tube labeled for the determinations of reduced glutathione (GSH), malondialdehyde (MDA), gamma aminobutyric acid (GABA) and GABA-transaminase (GABA-T). GSH was determined by the Ellman method [27], MDA was determined by the method described by Wilbur et al. [28], GABA concentration was determined by the method of Lowe et al. [29], and GABA-transaminase (GABA-T) activity was assessed by the colorimetric method of Nayak and Chatterjee [30].

2.3. Statistical analysis

The statistical analyses of the values obtained in this study were carried out using XLStat, Microsoft Office Excel 2010 software. Results were expressed as mean ± standard error over the mean (ESM). The different values were compared using the analysis of variance test (ANOVA). Where differences existed, the Newman Keuls multiple comparison test was used to separate them. Fisher's exact probability was used to compare the average percentages calculated from the experimental data. From $p \leq 0.05$, differences will be considered significant.

3. RESULTS

3.1. Phytochemical screening of *Ocimum gratissimum*

Table 1 shows the main groups of chemical molecules contained in the aqueous extract of *O. gratissimum* leaves. It appears that this extract contains alkaloids, anthraquinones, flavonoids, tannins, saponins, polyphenols and glycosides.

Table 1: Phytochemical screening of leaf extract of *Ocimum gratissimum*

Phytochemical	*Extract content*
Alkaloids	++
Anthraquinones	++
Flavonoids	+
Tanins	+++
Triterpenes	-
Saponins	+
Polyphenols	+++
Glucosides	+++

+ = Amount present; ++ = Moderate amount present; +++ = Appreciate amount present; - = Amount absent

3.2. Preventive effects of decoction of *O. gratissimum* on pilocarpine-induced *epilepticus status*

Figure 2 shows the effects of the decoction of *O. gratissimum* on the status epilepticus. It appears that the latency time of onset of status epilepticus is 20.45 min ($p<0.001$) in the negative control compared to the normal control in which this time is zero, proof of the establishment of the status epilepticus. *O. gratissimum* L. inhibited the onset of convulsions by increasing this time by 22.2, 22.79, 36.47, 39.83 min ($p<0.001$) in the treatment groups at doses of 24.4, 61, 122 and 244 of the decoction, respectively, compared to the negative control. Similarly, sodium valproate significantly increased this time to 51.17min ($p<0.001$) compared to the negative control.

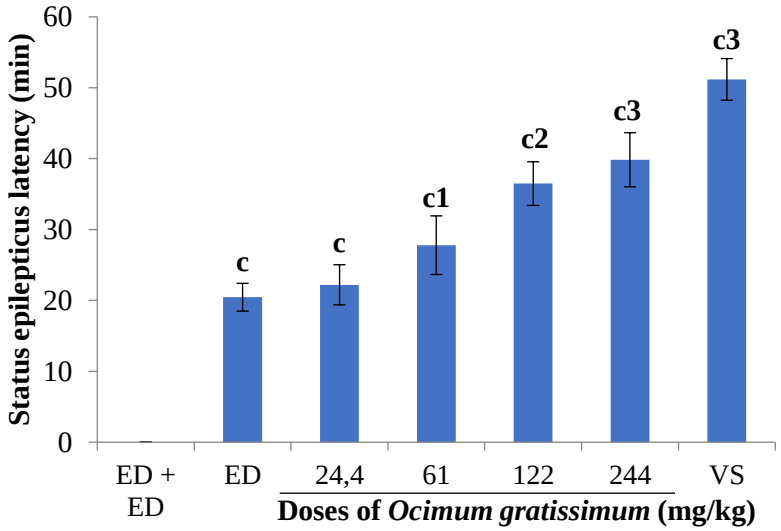

Figure 2: Preventive effects of decoction of *Ocimum gratissimum* on epilepticus status

Each bar represents the mean ± the MSE of the group, n = 6. cp<0.001; significant difference compared to normal control and 1p<0.05; 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: negative control, treated with distilled water, then pilocarpine; 24.4-244 mg/kg: Doses of the decoction of *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg), and given pilocarpine (360 mg/kg).

3.3. Curative effects of decoction of *O. gratissimum* on tonic and clonic seizures induced by pilocarpine

3.3.1. Effects of *O. gratissimum* decoction on pilocarpine-induced tonic and clonic seizure latency

Figure 3 shows the effects of the decoction of *O. gratissimum* on the latency time of onset of the first tonic or clonic convulsion. It appears that this time increased to 40.66 sec (p<0.001) in the negative control compared to the normal control in which it is zero. *O. gratissimum* delayed the onset of seizures by increasing this time in a dose-dependent manner to 275.5, 576.66, and 1128.33 sec (p<0.001) in the treatment groups at doses of 61, 122 and 244 of the decoction, respectively, compared to the negative control. Similarly, sodium valproate increased this time highly significantly, to 1567.83 s (p<0.001) compared to the negative control.

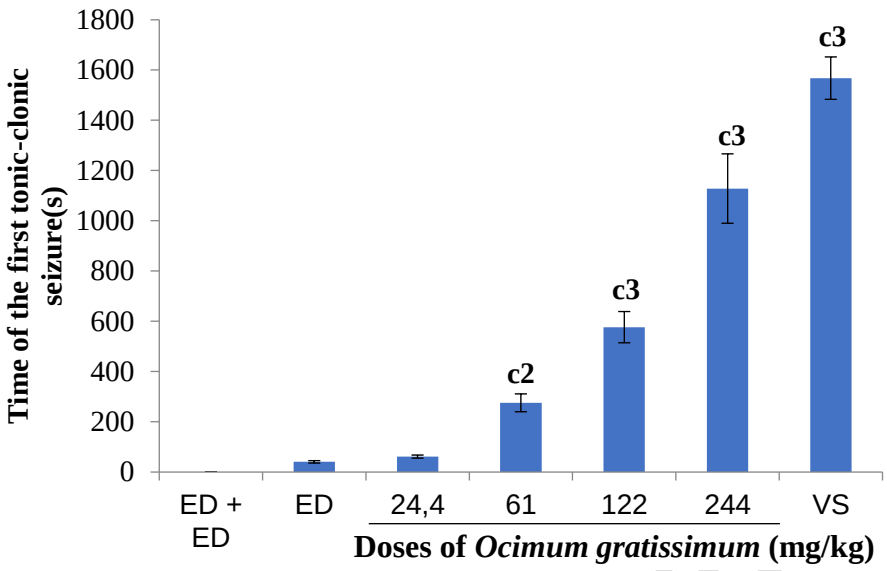

Figure 3: Effects of decoction of *Ocimum gratissimum* on the latency time of tonic and clonic seizures induced by pilocarpine

Each bar represents the mean ± the MSE of the group, n = 6. cp<0.001; significant difference compared to normal control and 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: negative control, treated with distilled water, then pilocarpine; 24.4-244: Doses of the decoction of *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg), and given pilocarpine (360 mg/kg).

3.3.2. Effects of decoction of *O. gratissimum* on the number and duration of clonic seizures induced by pilocarpine

Figure 4 summarizes the effects of a decoction of *O. gratissimum* on the number and duration of clonic seizures 24 hours after ES induction. It appears that the number of clonic seizures increased to 22.83 (p<0.001) in the negative control compared to the normal control in which no clonic seizures were observed. *O. gratissimum* induced a dose-dependent decrease in this number to 22.16; 15.66; 7.16; 3.66 in the groups treated at doses of 24.4; 61; 122 and 244 mg/kg of the decoction respectively; a reduction of 3; 31; 69 and 84% (p<0.001) compared to the negative control. Similarly, sodium valproate induced a significant decrease in this number to 3.33, or 85% (p<0.001) compared to the negative control.

The duration of clonic seizures decreases at a dose-dependent rate of 24.16; 15.83; 12.16; 10.33 sec in the groups treated at the respective doses of 24.4; 61; 122 and 244 mg/kg of the decoction, i.e. 36, 58, 68 and 73% (p<0.001) respectively compared to the negative control. Similarly, sodium valproate induced a decrease in the duration of clonic seizures to

6.33 sec, equivalent to 83% ($p<0.001$) compared to the negative control, in which it is 37.83 sec.

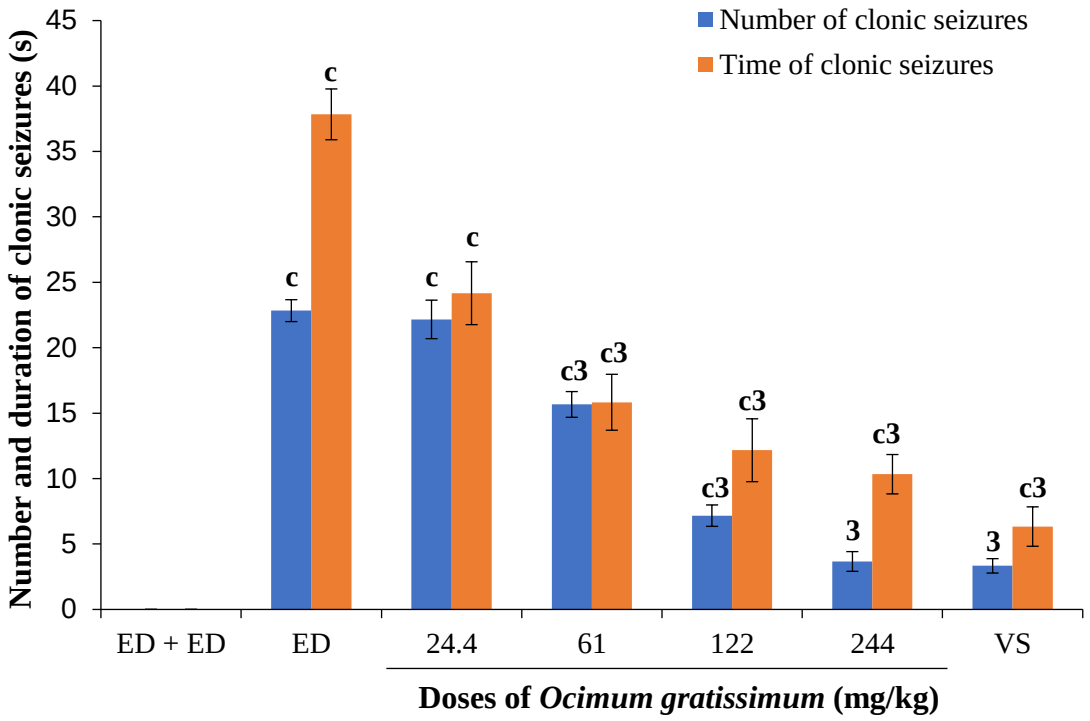

Figure 4: Effects of decoction of *Ocimum gratissimum* on the number and duration of clonic seizures

Each bar represents the mean ± the MSE of the group, n = 6. cp<0.001; significant difference compared to normal control and 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: negative control, treated with distilled water, then pilocarpine; 24.4-244 mg/kg: Doses of the decoction of *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg), and given pilocarpine (360 mg/kg).

3.3.3. Effects of decoction of *O. gratissimum* on the number and duration of pilocarpine-induced tonic seizures

Figure 5 summarizes the effects of *O. gratissimum* decoction on the number and duration of tonic seizures 24 hours after ES induction. It appears that the number of tonic seizures increased to 8.5 ($p<0.001$) in the negative control compared to the normal control in which no clonic seizures were observed. *O. gratissimum* induced a dose-dependent decrease in this number of 6.16; 5.83; 4.66; 3.83 in dose groups 24.4; 61; 122 and 244 of the decoction, a reduction of 27; 31; 45 and 55 ($p<0.001$)% respectively, compared to the negative control.

Similarly, sodium valproate induced a significant decrease in this number to 2.5 ($p<0.001$), a reduction of 71% compared to the negative control.

The duration of tonic seizures decreases dose-dependent to 12.66; 8.16; 7.16; 6.33 sec ($p<0.001$) in groups treated at doses 24.4; 61; 122 and 244 mg/kg of the decoction, equivalent to a reduction of 20, 46, 53 and 58% respectively, compared to the negative control. Similarly, sodium valproate induced a decrease in this duration to 3.16 sec; This is a reduction of 79% ($p<0.001$) compared to the negative control, in which it is 15.66s.

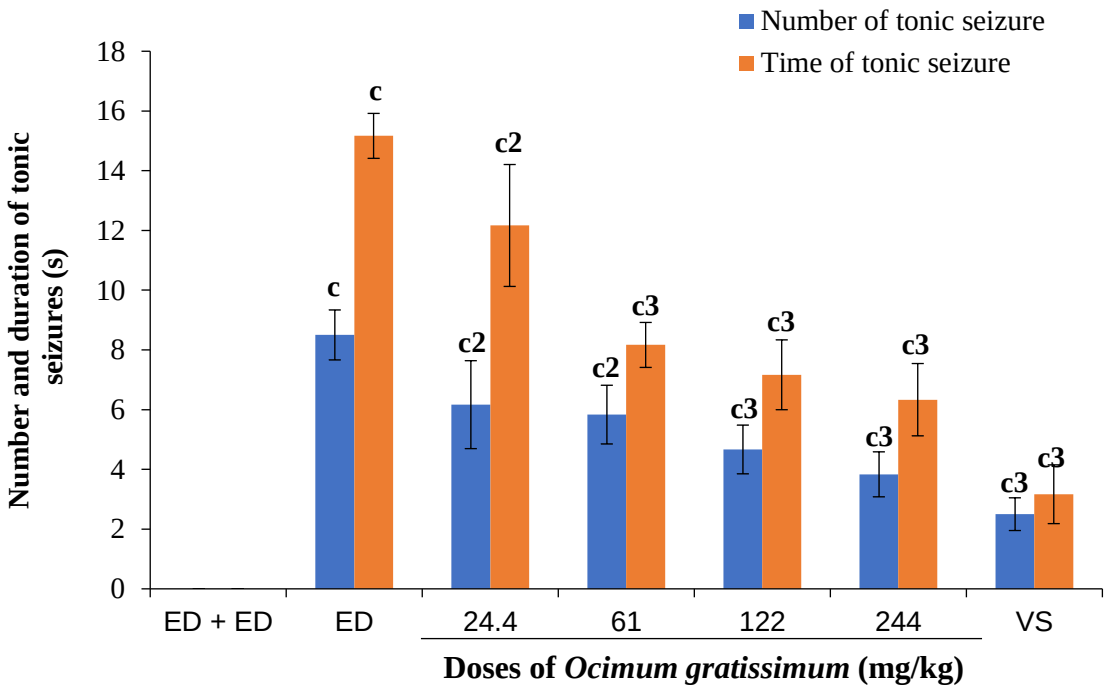

Figure 5: Effects of the decoction of *Ocimum gratissimum* on the number and duration of tonic seizure

Each bar represents the mean ± the MSE of the group, n = 6. cp<0.001; significant difference compared to normal control and 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: negative control, treated with distilled water, then pilocarpine; 24.4-244 mg/kg: Doses of the decoction of *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg), and given pilocarpine (360 mg/kg).

3.3.4. Effects of *O. gratissimum* decoction on pilocarpine-induced tonic and clonic seizure score

Figure 6 summarizes the effect of the decoction of *O. gratissimum* on the seizure score. It shows that the seizure score decreased by 100% ($p<0.001$) in the negative control compared to the normal control, ranging from 1 in the latter to 0 in the negative control. This score increased dose-dependent by 0.34, 0.85, 0.92, ($p<0.001$) in mice treated at 24.4, 61, and 122 mg/kg respectively compared to the negative control. The 244 mg/kg dose of decoctate and sodium valproate increased this score by 0.96 and 0.97 ($p<0.001$) respectively, compared to the negative control.

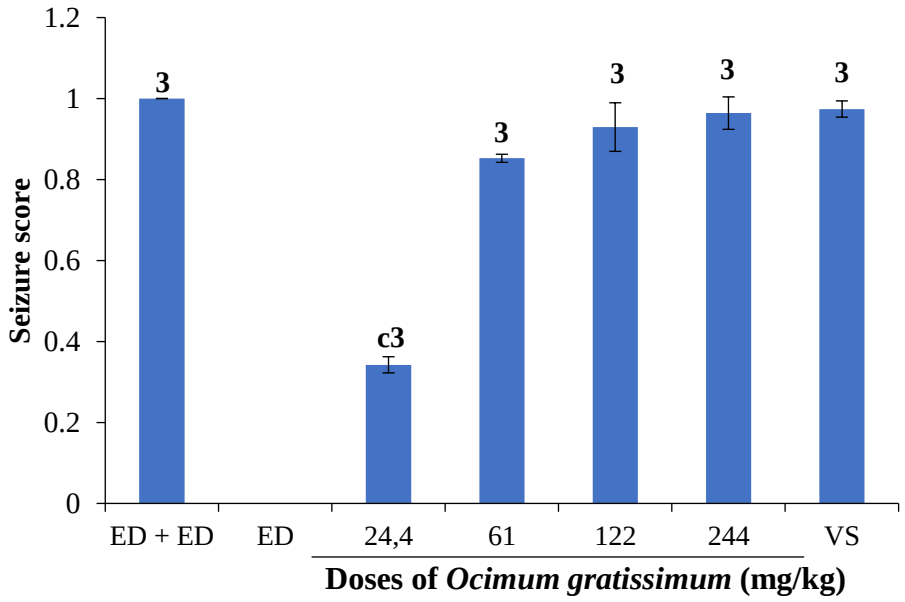

Figure 6: Effect of decoction of *Ocimum gratissimum* on the score of tonic and clonic seizures induced by pilocarpine

Each bar represents the mean ± the MSE of the group, n = 6 n = 6. cp<0.001; significant difference compared to normal control and; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and then diazepam; 24.4-244 mg/kg: Doses of the decoction of *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg).

3.4. Anxiolytic effects of decoction of *O. gratissimum* in mice made epileptic by acute administration of pilocarpine

3.4.1. Anxiolytic effects of the decoction of *O. gratissimum* in epileptic mice placed in the raised cross labyrinth

3.4.1.1. Effects of decoction of *O. gratissimum* on classical variables

Table 1 illustrates the effects of the decoction of *O. gratissimum* on the number of entries and time spent in open arms (OA), the number of entries and time spent in closed arms (CA),

the total number of entries (TE) and time spent in all arms of the raised cross labyrinth, the ratio OE/TE versus CE/TE; the number of 'head dippings', the number of 'rearing' and the number of entrances to the centre of the raised cross labyrinth.

a. Effect of decoction on the number of entries and time spent in open arms of the labyrinth

The number of open arms entries increased from 1.83 ± 0.27 in the normal control to 1.16 ± 0.27 in the negative control. *O. gratissimum* induced a dose-dependent increase of this number to 1.5 ± 0.66; 1.83 ± 0.55; and 2.33 ± 0.44 sec <0.001) in the treatment groups at respective doses of 61; 122 and 244 mg/kg of the decoctate, compared to the negative control. Similarly, sodium valproate and diazepam induced a significant increase in this number to 2.5 ± 0.66 and 3.16 ± 0.55 ($p<0.001$) respectively; compared to the negative control.

Time spent in open arms increased from 26.6 ± 3.33 sec normal control, to 17.83 ± 3.83 sec in the negative control. *O. gratissimum* induced an increase in this time to 45.83 ± 6.72; 78.16 ± 4.83; 166.33 ± 12.1 and 212.33 ± 6.55 sec ($p<0.0001$) in dose groups 24.4; 61; 122 and 244 mg/kg of the decoction respectively; compared to the negative control. Sodium valproate administered within the same time frame as decoctate also increased this time to 216.83 ± 34.27 sec ($p<0.001$). Similarly, diazepam increased this time to 236.66 ± 8.33 sec ($p<0.0001$) compared to the negative control.

b. Effect of the decoction of *O. gratissimum* on the number of entries and time spent in the closed arms of the labyrinth

The number of closed arms entries increased significantly in the negative control compared to the normal control, ranging from 7.83 ± 1.2 to 3.33 ± 0.66 in the normal control. This number decreased in a dose-dependent manner to 5.83 ± 0.88; 4.66 ± 1.33; and 2.66 ± 0.66 and 1.66 ± 0.66 ($p<0.0001$) in the groups treated at respective doses of 24.4; 61 and 122 and 244 mk/kg of decocted. Sodium valproate and diazepam induced a significant decrease in this number to 1.66 ± 0.66 and 1.66 ± 1.66 ± 0.27 ($p<0.001$) respectively, compared to the negative control.

Time spent in closed arms increased in the negative control compared to the normal control, ranging from 158.5 ± 12.16 sec in the latter to 194.83 ± 30.83 sec in the normal control. This time decreases significantly with dose to 118.16 ± 13.22; 37.16 ± 2.16 and 22.83 ± 2.16 mg/kg ($p<0.001$); in the groups treated at doses of 61; 122 and 244 mg/kg respectively, compared to the negative control. Sodium valproate induced a significant decrease in this time to 17.33 ± 1.57 sec ($p<0.001$) compared to the negative control, as well as diazepam, which

induced a significant decrease in this time, to 13.5 ± 1.66 sec ($p<0.001$) compared to the negative control.

c. Effect of *O. gratissimum* decoction on number of entries, time spent in all arms of the maze and ratio EO/ET versus EF/ET

The number of entries in all arms of the maze increases in the negative control (9 ± 1.33) compared to the normal control (5.16 ± 0.55). In groups treated at respective doses of 24.4; 61; 122 and 244 mg/kg, this number decreases in a dose-dependent manner to 7 ± 1; 6.16 ± 1.5; 4.5 ± 1.66; 4 ± 0.33 respectively, compared to the negative control where it was 9 ± 1.33. In addition, sodium valproate and diazepam both induced a significant decrease in this number to 4.16 ± 0.55; and 4.33 ± 0.77 ($p<0.001$) respectively, compared to the negative control.

The time spent in all arms of the maze, increased in the negative control compared to the normal control. It increased from 185.16 ± 12.88 sec in the normal control to 212.66 ± 30 sec in the negative control. This number increases at a dose-dependent rate of 181 ± 18.66 sec; 196.33 ± 9.44 sec; 203.5 ± 12.66 sec; and 235.16 ± 6.83 sec in the dose groups 24.4; 61; 122 and 224 mg/kg of the decoction. Sodium valproate induced an increase in this number to 234.16 ± 34.72 sec, as did diazepam which induced an increase of this time to 250.16 ± 9.16 sec, compared to the negative control where it was 212.66 ± 30 sec.

The ratio (number of entries in open arms (OE)/ total number of entries (TE) versus total number of entries in closed arms (CE) / total number of entries) decreases in the negative control in which it is 14.89, compared to the normal control in which it is 55. This ratio increases dose-dependent to 20; 32.14; 68.75; 140, in the groups treated respectively at doses of 24.4; 61; 122 and 244 mg/kg of the decoction. Sodium valproate induced a significant increase in this ratio to 140 ($p<0.001$) compared to the negative control, similarly, diazepam induced a significant increase in this number to 271.4 ($p<0.001$).

d. Effect of the decoction of *O. gratissimum* on the number of head dippings

Pilocarpine injection significantly increased the number of head dippings from 2.33 ± 0.77 in the normal control to 5.33 ± 0.57 in the negative control. This number decreases to 3± 1; 2.16 ± 0.88; 1.83 ± 0.55; and 1.33 ± 0.44 ($p<0.001$) respectively, in the treatment groups at respective doses of 24.4; 61; 122 and 224 mg/kg of the decoction compared to the negative control. Valproate and diazepam also induced a decrease in this number to 1.16 ± 0.27 and 0.66 ± 0.17 ($p<0.001$) respectively, compared to the negative control.

Time spent in closed arms increased in the negative control compared to the normal control, ranging from 158.5 ± 12.16 sec in the latter to 194.83 ± 30.83 sec in the normal control.

This time decreases significantly with dose to 118.16 ± 13.22; 37.16 ± 2.16 and 22.83 ± 2.16 mg/kg ($p<0.001$); in the groups treated at respective doses of 61; 122 and 244 mg/kg, compared to the negative control. Sodium valproate induced a significant decrease in this time to 17.33 ± 1.57 sec ($p<0.001$) compared to the negative control, as well as diazepam, which induced a significant decrease in this time, to 13.5 ± 1.66 sec ($p<0.001$) compared to the negative control.

e. Effect of the decoction of *O. gratissimum* on the number of 'rearing'

The number of rearings increased in the negative control compared to the normal control, ranging from 13.33 ± 0.85 in the negative control to 7.16 ± 1.33 in the normal control. *O. gratissimum* induced a decrease in this number to 7.33 ± 1.33; 5.16 ± 1.22; 2.66 ± 1.11 and 1.66 ± 0.66 ($p<0.001$) in the respective dose groups, 24.4; 61; 122 and 224 mg/kg of the decoction, compared to the negative control. Sodium valproate administered within the same time frame as the decoction decreased this number to 1.66 ± 0.66 ($p<0.0001$), as well as diazepam which decreased this number to 1.16 ± 0.27 ($p<0.0001$), compared to the negative control.

f. Effect of the decoction of *O. gratissimum* on the number of entrances to the center of the labyrinth

The number of entries to the center of the labyrinth decreased in the negative control compared to the normal control, from 2.83 ± 0.5 in the normal control to 1.66 ± 0.3 in the negative control. This number increases to 1.83±0.5; 2.5 ± 0.66; and 2.83 ± 0.61 in dose groups 61; 122 and 224 mg/kg of the decoction. Sodium valproate induced an increase in this number to 2.33 ± 0.77, as did diazepam which induced an increase to 2.66 ± 0.66, compared to the negative control.

Table 2: Anxiolytic effects of the decoction of *Ocimum gratissimum* in epileptic mice placed in the raised cross labyrinth

Groups	*Number of entries in OA*	*Time spent in OA (s)*	*Number of entries in CA*	*Time spent in CA (s)*	*Number of entries in all arms*	*Time spent in all the arm(s)*	*Ratio OE/TE versus CE/TE*	*Number of 'head dipping'*	*Number of 'rearing'*	*Number of entries to the centre*
ED+ED	1.83±0.27	26.6±3.33^{2}	3.33±0.66	158.5±12.16	5.16±0.55^{2}	185.16±12.88	55.00^{3}	2.33±0.77^{a}	7.16±1.04^{2}	2.83±0.5^{1}
ED	1.16±0.27	17.83±3.83^{b}	7.83±1.2^{b}	194.83±30.83	9.00±1.33^{b}	212.66±30	14.89^{c}	5.33±0.57^{1}	13.33±0.85^{b}	1.66±0.3^{a}
24.4	1.16±0.27	45.83±6.72^{b3}	5.83±0.88^{a}	135.16±15.22^{3}	7.00±1.00	181±18.66	20.00b2	3.00±1.00^{2}	7.33±1.33^{2}	1.33±0.44^{a}
61	1.5±0.66	78.16±4.83^{c3}	4.66±1.33^{2}	118.16±13.22^{b3}	6.16±1.5	196.33±9.44	32.14b2	2.16±0.88^{3}	5.16±1.22^{3}	1.83±0.5^{a}
122	1,83±0,55	166.33±12.1^{c3}	2.66±0.66^{3}	37.16±2.16^{c3}	4.5±1.66^{2}	203.5±12.66	68.75b3	1.83±0.55^{3}	2.66±1.11^{c3}	2.5±0.66^{1}
244	2.33±0.44^{b2}	212.33±6.55^{c3}	1.66±0.66^{3}	22.83±2.16^{c3}	4±0.33^{3}	235.16±6.83	140c3	1.33±0.44^{3}	1.66±0.88^{c3}	2.83±0.61^{1}
VS (300 mg/kg	2.5±0.66^{b2}	216.83±34.27^{c3}	1.66±0.66^{3}	17.33±1.55^{c3}	4.16±0.55^{3}	234.16±34.72^{c}	150c3	1.16±0.27^{3}	1.66±0.66^{c3}	2.33±0.77^{1}
Diaz (3 mg/kg)	3.16±0.55^{b2}	236.66±8.3^{c3}	1,66±0,27^{a3}	13,5±1,66^{c3}	4,33±0,77^{3}	250,16±9,16	271.4c3	0.66±0.66^{a3}	1.16±0.27^{c3}	2.66±0.66^{1}

Each value represents the mean ± the MSE of the group, n = 6. ap<0.05; bp<0.01; cp<0.001; significant difference from normal control and 1p<0.05 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: positive control treated with diazepam (3 mg/kg); 24.4-244 mg/kg: Doses of *O. gratissimum*; OA: Open Arm; CA: Closed Arm; OE: Entry in the open arm; CE: Entry in the closed arm; TE: Total Entry.

3.3.1.2. Effect of decoction of *O. gratissimum* on percentages of number of entries and time spent in open arms of the labyrinth

Figure 7 shows the percentages of entries and time spent in the open arms of the maze. It shows that the percentage of the number of entries in open arms decreased significantly from 35.48% ($p<0.001$) in the normal control, to 12.96% in the negative control. *O. gratissimum* induced a dose-dependent increase of 16.66; 24.32; 40.74; 58.33% ($p<0.001$) in dose groups 24.4; 61; 122 and 224 mg/kg of decocted. Sodium valproate induced an increase to 60%, as well as diazepam, for which the increase is 73.07% ($p<0.001$) compared to the negative control.

The percentage of time spent in the open arms of the labyrinth decreases significantly in the negative control in whom it is 5.94% compared to the normal control in which it is 8.88%. *O. gratissimum* induced a dose-dependent increase in this percentage in the dose groups 24.4; 61; 122 and 224 mg/kg of the decoction, where it is respectively 15.27; 26.07; 55.44; and 70.77% ($p<0.001$). Sodium valproate and diazepam induced an increase in this number to 72.27%, and 78.88% ($p<0.001$) respectively.

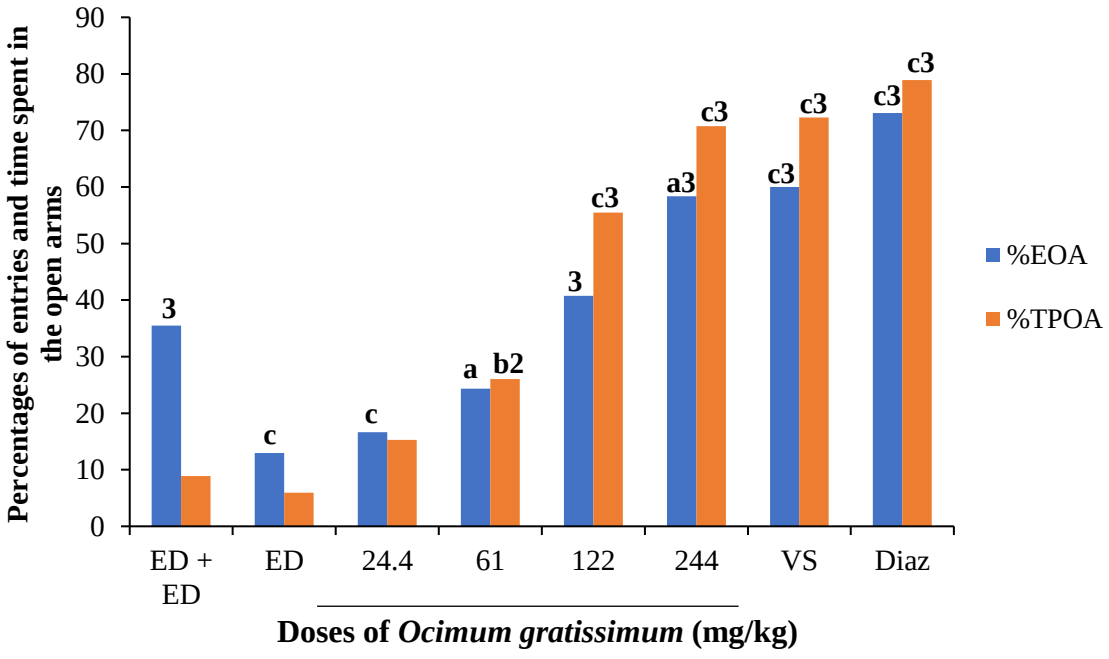

Figure 7: Effects of the decoction of *Ocimum gratissimum* on the average percentages of entries and time spent in the open arms of the labyrinth

Each bar represents the average percentage of the group, n = 6. ap<0.05; bp<0.01; cp<0.001; significant difference compared to normal control and 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; 24.4-224 mg/kg: Doses of the decoction of *O. gratissimum*;

VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: Positive control treated with diazepam (3 mg/kg).

3.3.1.3. Effect of decoction of *O. gratissimum* on percentages of time spent in closed arms of the labyrinth

Figure 8 shows the percentages of entries (%ECA) and the time spent in closed arms (%TPCA) of the maze. The mean percentage of time spent in closed arms increased significantly in the negative control compared to the normal control, ranging from 52.83% in the latter to 64.94% in the negative control. This percentage decreases with dose, by 45.05; 39.38; 12.38 and 7.61% ($p<0.001$) in the treatment groups at respective doses of 24.4; 61; 122 and 244 mg/kg of the decoctate, compared to the negative control. Sodium valproate induced a decrease in this percentage to 5.77% ($p<0.001$) compared to the negative control, as well as diazepam which induced a decrease in this percentage to 4.5% ($p<0.001$) compared to the negative control.

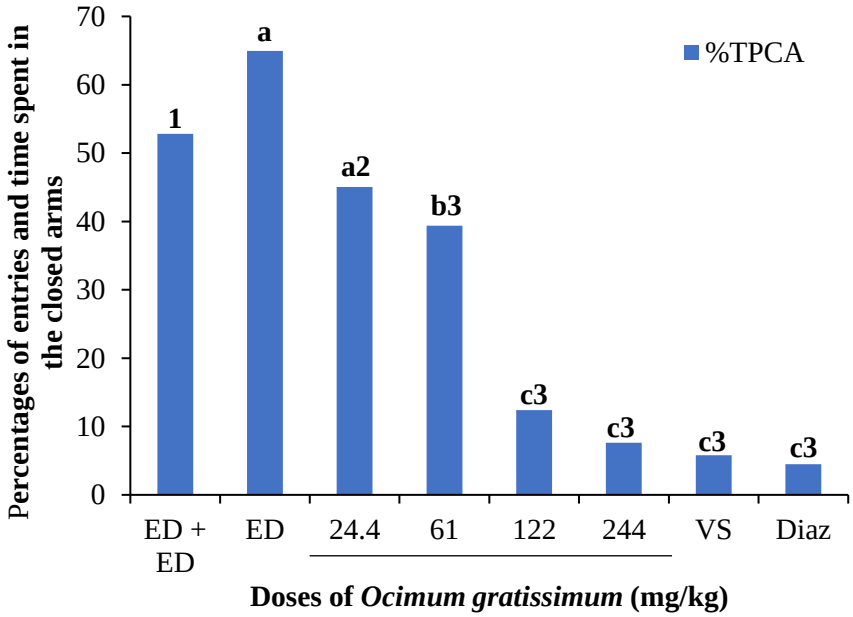

Figure 8: Effect of decoction of *Ocimum gratissimum* on the percentages of time spent in the closed arms of the labyrinth

Each bar represents the average percentage of the group, n = 6. ap<0.05; bp<0.01; cp<0.001; significant difference from normal control and 1p<0.05; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; 24.4-224 mg/kg: Doses of the decoction of *O. gratissimum*;

VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: Positive control treated with diazepam (3 mg/kg).

3.4.2. Anxiolytic effects of a decoction of *O. gratissimum* in epileptic mice placed in the open arena

Table 2 summarizes the effects of the decoction of *O. gratissimum* on the number of rearing, grooming and time spent in the center of the open arena. It shows that the number of "rearing" increased to 8.16 ± 0.55 ($p<0.001$) in the negative control compared to the normal control, in which it is 4.16 ± 0.83. *O. gratissimum* induced a decrease in this number to 5.16 ± 2.44; 3.83 ± 1.16; 2.66 ± 0.77 and 2.16 ± 0.55 ($p<0.001$) in the dose groups 24.4; 61; 122 and 244 mg / kg of the decoction respectively; compared to the negative control; i.e. a decrease of 73% induced by the dose 244 mg/kg. Sodium valproate also induced a decrease in this number to 1.83 ± 0.83 ($p<0.001$), or 78% compared to the negative control, and diazepam decreased this number to 1.66 ± 0.44 ($p<0.001$), or 80% compared to the negative control.

The number of crossings increased significantly in the negative control compared to the normal control, ranging from 16.16 ± 1.6 in the latter to 11.33 ± 1.33 in the negative control. *O. gratissimum* L. induced a dose-dependent increase in this number to 15.83 ± 2.5; 18.5 ± 0.66; 21.16 ± 0.68 in the dose groups 24.4; 61; 122 mg/kg respectively. The dose 224 mg/kg of the decoction increased it to 23.16 ± 1.83; or 104% ($p<0.001$) compared to the negative control. Sodium valproate also increased this number to 22.33 ± 2.33 ($p<0.001$), and diazepam induced an increase to 26.83 ± 1.83 ($p<0.001$) compared to the negative control.

The number of groomings decreased non-significantly in the negative control compared to the normal control. It went from 3.16 ± 2.94 to 0.83 ± 0.27 in the negative control. This number increased to 1.5 ± 0.5 in mice treated with 24.4 and 122 mg/kg, and to 1.5 ± 0.66 in those treated with 61 mg/kg of the decocted *O. gratissimum*. The dose 244mg/kg increased this number to 2, or 140% compared to the negative control. Sodium valproate and diazepam induced an increase to 2.5 ± 0.66 and 2.33 ± 0.44 compared to the negative control, in which it is 0.83 ± 0.27.

The time spent in the center of the open arena decreased in the negative control compared to the normal control. It went from 4.83 ± 0.55 sec to 2.16 ± 0.2 sec in the negative control. The decoction of *O. gratissimum* induced a dose-dependent increase of this time to 7.16 ±1.1; 12.66 ±1.1; 19.5 ± 2.6 and 23.16 ± 0.8 in mice treated at doses 24.4; 61; 122 and 224 mg/kg respectively, i.e. 80% for doses 24.4-122 mg/kg, and 140% ($p<0.001$) for dose 244 mg/kg, compared to the negative control. This time also increased significantly to 24.33 ± 0.7

and 29.33 ± 2.16 (p<0.001) in mice treated with sodium valproate and diazepam, respectively, compared to the negative control.

Table 3: Effects of decoction of *Ocimum gratissimum* on anxiety parameters in the open arena

Groups	*Number of 'crossing'*	*Number of 'rearing'*	*Number of 'grooming'*	*Time spent at the centre (sec)*
ED+ED	16.66 ± 1.66[1]	4.16 ± 0.83[2]	3.16 ± 2.94[1]	4.83 ± 0.55
ED	11.33 ± 1.33[a]	8.16 ± 0.55[b]	0.83 ± 0.27[a]	2.16 ± 0.2
24.4	15.83 ± 2.5	5.16±1.44[3]	1.5 ± 0.5	7.16 ± 1.1[b3]
61	18.5 ± 0.6[3]	3.83 ± 1.16[3]	1.5 ± 0.66	12.66 ± 1.1[c3]
122	21.16 ± 0.8[b3]	2.66 ± 0.77[3]	1.5 ± 0.5	19.5 ± 2.6[c3]
244	23.16 ± 1.83[c3]	2.16 ± 0.55[2]	2 ± 0.33[1]	23.16 ± 0.83[c3]
VS	22.33 ± 2.3[b3]	1.83 ± 0.8[a3]	2.5 ± 0.66[1]	24.33 ± 0.7[c3]
Diaz (0.3mg/kg)	26.83 ± 1.83[c3]	1.86 ± 0.44[a3]	2.33 ± 0.44[1]	29.33 ± 2.16[c3]

Each value represents the mean ± the MSE of the group, n = 6. ap<0.05; bp<0.01; cp<0.001; significant difference from normal control and 1p<0.05; 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; 24.4-244 mg/kg: doses of the decocted *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: Positive control treated with diazepam (0.3 mg/kg).

3.4.3. Effects of the decoction of *O. gratissimum* on some parameters of oxidative stress in the hippocampus of mice made epileptic by acute administration of pilocarpine

3.4.3.1. Effects of decoction of *O. gratissimum* on glutathione and malondialdehyde concentration

MDA levels increased by 210% (p<0.001) in the negative control compared to the normal control, from 130.33 ± 5.33 nmol/g tissue to 403.83 ± 2.83 nmol/g tissue in the negative control (Table 3). The decoction of *O. gratissimum* L. induced a dose-dependent decrease in this amount of MDA to 381.16; 303.33; 237.83 nmol/g tissue (p<0.01) in mice treated at doses 24.4; 61 and 122 mg/kg respectively, and at 131.5 nmol/g tissue (p<0.001) in the 244 mg/kg versus negative control group. Sodium valproate and diazepam also induced a decrease in MDA to 128.33 and 131.66 mol/g tissue (p<0.001) respectively, compared to the negative control.

The reduced glutathione level decreased by 48% ($p<0.001$) in the negative control compared to the normal control, from 206.66 ± 15.22 mol/g tissue to 108.33 ± 4.33 mol/g tissue in the negative control. The decoction of *O. gratissimum* L. induced a dose-dependent increase in this amount to 120.16, 144.66, and 168.16 mol/g tissue ($p<0.05$) in mice treated at doses of 24.4; 61 and 122 mg/kg ($p<0.001$) respectively; and 193.16 mol/g tissue in mice treated with 244 mg/kg, compared to the negative control. The same is true for sodium valproate and diazepam which induced a significant increase in the amount of glutathione reduced from 194.16 and 200.83 mol/g of tissue ($p<0.001$) compared to the negative control.

Table 4: Effects of decoction of *Ocimum gratissimum* on glutathione and malondialdehyde concentration

Groups	*Malondialdéhyde (nmol/g de tissu)*	*reduced Glutathione (mol/g de tissu)*
ED+ED	130.33 ± 5.33^{3}	206.66 ± 15.22^{3}
ED	403.83 ± 2.83^{c}	108.33 ± 4.33^{c}
24.4	381.16 ± 3.88^{c}	120.16 ± 1.27^{c}
61	303.33 ± 38.66^{c2}	144.66 ± 3.33^{c2}
122	237.83 ± 7.16^{c3}	168.16 ± 6.55^{c3}
244	131.5 ± 6.83^{3}	193.66 ± 5.11^{3}
VS (300 mg/kg)	128.33 ± 1.33^{3}	194.16 ± 4.44^{3}
Diaz (0.3mg/kg)	131.66 ± 5.77^{3}	200.83 ± 2.16^{3}

Each value represents the mean ± the MSE of the group, n = 6. AP<0.05; bp<0.01; cp<0.001; significant difference from normal control and 1p<0.05; 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; 24.4-244 mg/kg: doses of the decocted *O. gratissimum*; VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: Positive control treated with diazepam (0.3 mg/kg).

3.3.3.2. Effects of the decoction of *O. gratissimum* on gamma aminobutyric acid and on the activity of gamma aminobutyric acid transaminase in the hippocampus of mice made epileptic by acute administration of pilocarpine

GABA levels decreased in the negative control by 33% ($p<0.001$) compared to the normal control, from 389.33 ± 5.6 µg/g tissue to 261.16 ± 8.44 µg/g tissue in the negative

control (Table 4). The decoction of *O. gratissimum* induced a dose-dependent increase in the amount of GABA to 286.66; 312.33 and 330.66 µg/g tissue ($p<0.001$) in mice treated at doses of 24.4; 61; 122 mg/kg respectively; and 390.33 µg/g tissue ($p<0.001$) in those treated with 244 mg/kg, compared to the negative control. The same is true for sodium valproate and diazepam, which significantly increased this amount to 391.33 and 393.83 µg/g tissue (<0.001) respectively, compared to the negative control.

GABA-T activity increased by 114.1% ($p<0.001$) in the negative control compared to the normal control. The amount of GABA-T decreased from 50.33 ± 3.33 pg/min/mg tissue in the positive control to 107.66 ± 6.22 pg/min/mg tissue in the negative control. The decoction of *O. gratissimum* induced a dose-dependent decrease in this amount to 94.66 and 77.66 pg/min/mg tissue ($p<0.001$) in mice treated at respective doses of 24.4, 61 mg/kg. Doses 122 and 244 mg/kg reduced the amount of GABA-T to 59.16 and 53.33 pg/min/mg tissue ($p<0.001$) respectively, compared to the negative control. Sodium valproate and diazepam also induced a significant decrease in this amount to 54.83 and 55.16 pg/min/mg tissue ($p<0.001$) compared to the negative control.

Table 5: Effects of decoction of *Ocimum gratissimum* on GABA and GABA-transaminase activity

Groups	*GABA (µg/g of tissu)*	*GABA-T (pg/min/mg of tissu)*
ED+ED	389.33 ± 5.66	50.33 ± 3.33
ED	261.16 ± 8.44	107.66 ± 6.22
24.4	286.66 ± 8.33	94.66 ± 1.11
61	312.33 ± 1.11	77.66 ± 6.22
122	330.66 ± 10.44	59.16 ± 3.22
244	390.33 ± 4.33	53.33 ± 10.77
VS (300 mg/kg)	391.83 ± 4.16	54.83 ± 6.77
Diaz (0.3mg/kg)	393.83 ± 1.88	55.16 ± 3.22

Each value represents the mean ± the MSE of the group, n = 6. ap<0.05; bp<0.01; cp<0.001; significant difference from normal control and 1p<0.05; 2p<0.01; 3p<0.001 compared to negative control. ED+ED: Normal control, treated with distilled water; ED: Negative control, treated with distilled water and pilocarpine; 24.4-244 mg/kg: doses of the decocted *O.*

gratissimum; VS: Positive control treated with sodium valproate (300 mg/kg); Diaz: Positive control treated with diazepam (0.3 mg/kg).

4. Discussion

The anticonvulsant and anxiolytic activities of the decocted *O. gratissimum* were evaluated by tests of seizures and anxiety induced by pilocarpine. The results showed that the decoct of *O. gratissimum* significantly inhibits pilocarpine-induced seizures and anxiety in white mice. In the present study, inhibition of seizure number induced significant anticonvulsant activity of the decoct of *O. gratissimum.*

Intraperitoneal injection of pilocarpine is followed by its action on the muscarinic M1 receptor, causing an imbalance between excitatory and inhibitory synaptic transmission, resulting in the genesis of status epilepticus [31, 32]. The pilocarpine test consisted of causing epileptic seizures in mice, and observing important parameters for the evaluation of the preventive and anticonvulsant effect of the decoctate; in particular the latency time of the status epilepticus, then that of the first tonic or clonic seizure 24 hours after intraperitoneal injection of pilocarpine and the number and duration of tonic and or clonic convulsions. It is suggested that the longer the latency time, the duration and the number of seizures reduced, the more the plant has an antiepileptic effect [33]. The anticonvulsant activity of decoctate was therefore observed at a dose of 244 mg/kg, comparable to sodium valproate administered at a dose of 300 mg/kg, which attests to the antiepileptic activity of *O. gratissimum*.

Behavioral tests involve observing the animal's behavior in the raised cross maze and in the open arena. Decoction of *O. gratissimum.* significantly reduces the number of entries and the percentage of time spent in the closed arms of the labyrinth, as well as their percentages. Assuming that decreased activity in closed arms indicates a reduction in stress [34] and that an increase in activity in open arms shows a reduction in anxiety in the maze [35, 36, 37, 38], the decoct of *O. gratissimum* has anxiolytic properties. This reduction of anxiety would therefore be done by action on the sites of benzodiazepines of the GABA receptor complex and or by antagonism of 5-HT 2 and 5-HT 3 receptors [39]. It also appears from our study that the decoct of *O. gratissimum* significantly and dose-dependent reduces the number of 'rearing' both in the closed arms of the labyrinth and in the open arena. The significant decrease in the number of head-diping in mice given 224 mg/kg *O. gratissimum* is comparable to the reduction induced by diazepam at 3 mg/kg. These results are consistent with the observations of Rodgers et al., and Augustsson [38,40], according to which, an decrease in the number of 'rearing' and 'head dipping' respectively in the closed and open arms of the labyrinth indicates a decrease in

anxiety in rodents. This behavioural redistribution of mice could logically be attributed to an anxiolytic consequence rather than to behavioural non-specificity [38]. Thus, our results suggest that the decoct of *O. gratissimum* would possess anxiolytic properties. These properties are thought to be mediated by GABAergic neurotransmission in the cerebral cortex and hippocampus [35, 37, 38].

The open arena test makes it possible to evaluate several behavioural parameters in mice in response to a new environment or in the presence of an anxiety-provoking component [35, 41]. The results obtained during the open arena test showed a significant and dose-dependent increase in the number of crossings in mice treated with *O. gratissimum* decoctate. The decoct at the dose 244 mg/kg significantly increases the parameters of time spent at the center, the number of 'grooming', and the number of 'crossing'; This is due to the increase in locomotor activity and the level of exploration in rodents. This reflects an intrinsic manifestation of anxiety reduction [37, 39].

Oxidative stress parameters were evaluated using malondialdehyde (MDA), reduced glutathione, GABA and GABA-Transaminase assays. Neurochemical and enzymatic studies have suggested that pilocarpine-induced excitotoxic activity contributes to the production of reactive oxygen species (ROS); responsible for oxidative stress. Once released into tissues, free radicals (RLs) induce membrane damage and cell death through mechanisms such as protein oxidation [42] or lipid peroxidation [43]. It has been reported that the hippocampus of animals given pilocarpine and with status epilepticus shows increased levels of hydroperoxides; by-products of lipid peroxidation [44]. In the present study, *O. gratissimum* induced a significant decrease in MDA levels, a marker of peroxidation in the hippocampus of mice, as well as an increase in reduced glutathione levels. Substances capable of antagonizing the activity of lipid peroxidation markers in the cell are antioxidants [45]. These results suggest antioxidant activity of *O. gratissimum* that could explain the anticonvulsant effects of decocted, and are confirmed by those of Ikeotuonye et al., and Fofie et al., who report that, *O. gratissimum* has a strog antioxydant activity, due to the presence of phenlic compounds, flavonoids and alkaloids [46, 47].

According to the work of Gale [48], the inhibition of GABAergic neurotransmission would be at the origin of the appearance of seizures, while the facilitation of its neurotransmission by blocking the activity of GABA-T contributes to inhibit them. This anticonvulsant property of *O. gratissimum* therefore suggests an effect on the transmission of GABA, which is the main inhibitory neurotransmitter of the brain, very involved in epilepsy [49]; which is justified by the significant increase in the level of GABA and the decrease in the

level of GABA-transaminase in the hippocampus of mice treated with the decoctate. Sodium valproate induces the increase in cerebral GABA by inhibition of semialdehyde succinyl dehydrogenase or by activation of GABA synthesis by glutamic acid decarboxylase [49].

The qualitative phytochemistry of the decoction of the leaves of *O. gratissimum* revealed the presence of tanins, polyphenols, glucosides, alkaloids, anthraquinones, flavonoids and saponins. Secondary metabolites have been shown to be responsible for the biological activities of medicinal plants [50]. Alkaloids possess analgesic and sedative properties [51, 50]; and sedative drugs such as clonazepam have anticonvulsant effects [52, 53]. Flavonoids act on the modulation of GABAergic system by the GABA-A receptor, and the reduction of neuronal excitability by blocking voltage-gated NA+ channels [54, 55]. They also have anxiolytic properties due to their affinity for benzodiazepine site of the GABA receptor [56] ; phenolic compounds reduce the number and duration of seizures in the electrical model of induced temporal seizures in rats [57]. With Saponins, and alkaloids, they have anticonvulsivant properties by blocking voltage-dependant NA+ channels, or reducing their openig time [57, 58]. Furthermore, they have antioxydant properties with tanins [57, 58]. The presence of these compounds in the extract of *O. gratissimum* could therefore justify the previously reported effects.

5. Conclusion

All the work carried out in this study revealed that the decoction of the leaves of *O. gratissimum* effectively opposes the harmful effects of pilocarpine. It follows that, *O. gratissimum* has anticonvulsant and anxyolitic properties. These results therefore contribute to many pharmacological arguments in favor of the use of the decoction of this plant in traditional medicine as an alternative treatment for epilepsy and anxiety, and suggests a possible use of *O. gratissimum* as a preventive measure against epilepsy, particularly that of the temporal lobe. Further studies can be conducted to identify and characterise the active ingredients and their mechanism of action.

Disclaimer (artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Consent

It is not applicable.

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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