Anticonvulsant and Anti-amnesic Effects of an Aqueous Extract of *Crassocephalum bauchiense* (Hutch.) Milne-Redh. (Asteraceae) in Mice Pilocarpine Model of Temporal Lobe Epilepsy

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ABSTRACT

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| This study aimed to investigate the anticonvulsant and anti-amnesic effects of *Crassocephalum bauchiense* aqueous extract in pilocarpine model of temporal lobe epilepsy. Mice were treated for seven consecutive days as follows: one normal group and one negative control group that received orally distilled water; four test groups that received orally four doses of *Crassocephalum bauchiense* aqueous extract (28, 70, 140 and 280 mg/kg), respectively; and one positive control group that received intraperitoneally 300 mg/kg sodium valproate. One hour after the first treatment (first day), *status epilepticus* was induced by intraperitoneal injection of a single dose of pilocarpine (360 mg/kg). Twenty-three hours after the injection of pilocarpine to mice, they received once again their different treatments. Sixty minutes later, they were injected with a sub-convulsive dose of picrotoxin (1 mg/kg), and the anticonvulsant property of the extract was determined. On day-seven, T-maze, elevated plus maze and open field tests were performed. Finally, mice were sacrificed and the hippocampus were isolated to determine the GABAergic/cholinergic signaling in the brain of mice. The aqueous extract of *Crassocephalum bauchiense* increased significantly the latency time of the onset of the tonic and clonic seizures, decreased the number and duration of seizures. *Crassocephalum bauchiense* extracts (280 mg/kg) significantly increased the latency time to *status epilepticus* and strongly prevented the convulsions. Its significantly decreased the number of clonic and tonic seizures, and their duration, respectively. *Crassocephalum bauchiense* (140 and 280 mg/kg) also prevented status epilepticus induced cognitive impairment in mice. The extract also ameliorated the GABAergic and cholinergic neurotransmission. These results suggested that *Crassocephalum bauchiense* extract has anticonvulsant and anti-amnesic effects. The aqueous extract of *Crassocephalum bauchiense* ameliorated epileptogenesis of temporal lobe epilepsy and could be used for the treatment of temporal lobe epilepsy. |

*Keywords: Crassocephalum bauchiense*, temporal lobe epilepsy, anti-amnesic, anticonvulsant.

1. INTRODUCTION

“Epilepsy is a brain disorder characterized by recurrent and spontaneous seizures due to hyperexcitability and hypersynchronization of neurons, widely accepted as two or more seizures at least 24 hours apart” (Katyayan and Diaz-Medina, 2021). Approximately 70 million people are currently living with epilepsy worldwide and 80% of these people live in developing countries (Ngugi et *al.*, 2010; Espinosa-Jovel et al.*,* 2018). “Among the different forms of epilepsy, temporal lobe epilepsy is the most common and is often associated with anxiety, depression and cognitive impairment” (Gröticke et *al.*, 2008)**.**  Approximately 65% of patients with temporal love epilepsy (TLE) have cognitive and mainly memory problems (Oddo et al*.*, 2003 ; Faure et al., 2014). Memory disorders are associated with mesial TLE based on the role of the temporal lobe in the memory process (Faure et al., 2014). Risk factors such as stroke, neurocysticercosis, neuromalaria, onchocerciasis (Onchocercas volvulus), human African trypanosomiasis and bilharzia are particularly epileptogenic and can cause seizures or subsequent epilepsy at all ages of life (Dongmo et al., 2004). Mesial TLE is considered to be the most severe because 70% of its seizures are refractory to drug treatment (Lignelet, 2011). Several hypotheses surround this resistance and one of the unavoidable hypotheses is the loss of certain receptors and/or ion channels, which results from the significant cell death associated with hippocampal sclerosis and on which most anti-epileptic drugs usually act (Curia et al., 2008 ;Bressand et al., 2009). In some cases, the best results were obtained when anti-epileptic drugs were administered together following head trauma (Bayham et al., 2020). At the molecular level, in addition to the imbalance between GABAergic and glutamatergic neurotransmitters, epilepsy is linked to inflammation and oxidative stress. Experimentally, pilocarpine administration induces status epilepticus (SE) which in turn initiates epileptogenesis that may last one to two weeks (Blanco et al., 2009). Since pathophysiology emphasizes neuronal dysfunction and damage, and some patients present seizures that are refractory to available medications (Devinsky et al*.*, 2013), it is important to turn to nature to design a more effective therapy.

To treat this disease more effectively, it would be necessary to find drugs that are more tolerable, have fewer side effects, are accessible to all social classes and act on epileptogenesis (Leite et al*.*, 2002). Therefore, it is essential for scientific researchers to turn to medicinal plants, given that more than 80% of the population of developing countries uses them to alleviate various health problems (El Hilaly et al., 2004).

“The interest of our study is focused on *Crassocephalum bauchiense* of the Asteraceae family, which is a medicinal plant known for its beneficial effects in Cameroonian traditional medicine in the treatment of many disorders including gastrointestinal infestations, infantile convulsions, epilepsy and anxiety” (Mouokeu et al., 2011). “Previous studies have shown an antinociceptive activity (Taiwe et *al.*, 2012a), as well as antipsychotic and sedative effects” (Taiwe et al., 2012b) of the aqueous extract of *Crassocephalum bauchiense* leaves. Other works have shown that the ethyl acetate extract of *Crassocephalum* *bauchiense* leaves has antibacterial effects (Mouokeu et al., 2011), antifungals and antioxidants (Mouokeu et al., 2014) and immunomodulatory activity (Mouokeu et al., 2013). In addition, a preliminary phytochemical analysis of the aqueous extract prepared from the leaves of *Crassocephalum* *bauchiense* revealed the presence of phenols, alkaloids, flavonoids, tannins, triterpenes and sterols (Moukeu et al., 2011). The present study was designed to evaluate the anticonvulsant and anti-amnesic effects of an aqueous extract of *Crassocephalum bauchiense* in pilocarpine model of temporal lobe epilepsy.

2. material and methods

**2.1. Plant material and extract preparation**

*Crassocephalum bauchiense*, was collected during the rainy season (June) in Ngaoundere (Adamaoua Region, Cameroon). It was then identified at the National Herbarium of Cameroon (Yaoundé) by comparison with the sample registered under number 7954/SRF/Cam. The stems and leaves of *Crassocephalum bauchiense* were washed with water and then air-dried (in the shade) at room temperature. They were then crushed into a fine dry powder. The aqueous extract of *Crassocephalum bauchiense* was prepared by imitating the method of traditional practitioners. A decoction of the plant was prepared by introducing 10 g of dry powder of *Crassocephalum bauchiense* into a beaker containing 50 mL of distilled water. The mixture was boiled for 20 min on a hot plate at 100°C. After cooling, the mixture was filtered through a Whatman number 1 paper. The filtered mixture was 32 mL, and then the water was evaporated in an oven (50°C). The process yielded 0.9 grams of dry extract of *Crassocephalum bauchiense*, a yield of 9%. From the 28 mg/mL stock solution, i.e. 280 mg/kg, the different doses necessary for the experiment were diluted with distilled water to 1/2, 1/4, and 1/10, i.e. 140 mg/kg, 70 mg/kg and 28 mg/kg respectively. These doses were administered to the animals orally.

**2.2. Chemical products**

Pilocarpine, methyl-scopolamine, sodium valproate, piracetam were purchased from Sigma Chemical Co (St. Louis, USA) and picrotoxin from Roche, Paris, France). All reagents used for the determination of GABA, GABA-transaminase (GABA-T), acetylcholinesterase (AchE) and oxidative stress markers were obtained from Sigma Chemical Co (St. Louis, USA).

**2.3. Animals**

White mice, *Mus* *musculus* swiss, aged 31 to 47 days, of male sex weighing between 20 and 25 g were used in our experiments. The animals were provided by the National Veterinary Laboratory (LANAVET) of Garoua (North - Cameroon). All animals were acclimatized for 72 hours at the laboratory of medicinal plants, health and galenic formulation of the University of Ngaoundere before the beginning of the experiments. The mice were kept in Plexiglas cages and had free access to water. All experimental procedures in this study 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).

**2.4. Induction of *status epilepticus* and the study of anticonvulsant property**

On the first day of the experiments, 42 white *Mus musculus* Swiss mice were weighed and divided into 7 groups of 6 animals, as follows: - Four test groups received the different doses of *Crassocephalum bauchiense*(28, 70, 140 and 280 mg/kg *per os*); - One negative control group received distilled water (DW :10 mg/kg *per os*); - A positive control group received sodium valproate (VS: 300 mg/kg, i.p.); - A normal control group received distilled water (DW:10 mg/kg *per os*). The injection protocol was similar to those previously described by Turski et al*.,* (1983), Curia et *al*., (2008) and Magnin, (2014). Forty minutes after these administrations, a single low dose of N-methyl-scopolamine (1 mg/kg, i.p.) was administered to groups of animals except the normal group in an attempt to reduce the cholinergic effects of pilocarpine in the periphery. Twenty minutes after this injection, the mice received a single injection of pilocarpine (360 mg/kg, i.p.), a muscarinic cholinergic agonist, via the same route. The normal control received neither N-methyl-scopolamine nor sodium valproate. Then they were returned to their cages and observed individually for 6 hours the initial state of malaise called status epilepticus (SE) according to the Racine scale ranging from 0 to 5 stages: - Stage 0: no response; - Stage 1: hyperactivity and vibrissae clonus; - Stage 2: head nodding, head clonus and myoclonic jerks; - Stage 3: unilateral forelimb clonus; - Stage 4: bilateral forelimbs elevation and clonus; - Stage 5: tonic-clonic seizures with loss righting reflex, (Racine, 1972). The parameter as the latency time of status epilepticus was recorded. Mice that reached stage 5 according to the Racine scale were retained for further experiments.

*Excitus* and convulsions were induced by picrotoxin in mice 24 hours after the acute pilocarpine-induced *status epilepticus* test. This spontaneous neuropathology was facilitated by intraperitoneal injection of a sub-convulsive dose of picrotoxin (1 mg/kg) to mice. Briefly, animals were treated for the second day, or twenty-three hours after the injection of pilocarpine, with distilled water for group 1 and 2, the respective doses of the extracts for group 3 – 6, and sodium valproate for group 7, respectively. One hour later, a sub-convulsive dose of picrotoxin (1 mg/kg) was injected intraperitoneally to mice (groups 2 to 7), except group 1 that was injected intraperitoneally with saline. Each animal was observed immediately for a period of 30 min, and the incidence of seizures (the latency time to first clonic seizure, latency time to first tonic seizure, the number clonic seizures, duration of clonic seizures, number tonic seizures, and the duration of tonic seizures) were noted. Tonic-clonic seizures involve both tonic (a sudden stiffness or tension in the muscles of the arms, legs or trunk) and clonic (twitching or hock-like jerks of a muscle or a group of muscles) phases of muscle activity. The latency of tonic-clonic seizures was used to determine the seizure score. This score was calculated according to the following formula: Score = 1- negative control group latency/test group or positive control group latency.

Seven days after the previous study, the mice were used for the evaluation of the anti-amnesic effects of the plant. Another group of mice was added and served as a positive control for the memory test, and given orally piracetam (15 mg/kg, i.p.). The first test was the T-maze test, followed by the elevated plus maze test and the open field test. At the end of these behavioural tests, mice were sacrificed by cervical dislocation. Their brain and then the hippocampus were collected for the evaluation of the biochemical parameters.

**2.5. Behavioral analysis**

2.5.1. T-Maze Test

The T-maze test relies on the propensity of mice without cognitive decline to explore the new arm. This new arm is identified as the one without food (Deacon and Rawlins, 2006). The device is T-shaped and made from wood. This device is composed of a perpendicular arm opposite two closed arms. Each arm measured 30 cm × 10 cm × 20 cm (length × width × height) (Taiwe et *al.*, 2015). The T-maze test consisted of three phases: familiarization, acquisition or learning and retention. A black plastic cup (3 cm diameter, 1 cm deep) containing conventional mouse food was placed at the end of the two opposing arms.

On the first day (familiarization phase), the animals were introduced into the maze to explore it for 10 min. The animal was then allowed to choose either arm of the device indicating its preference. The arm in which the mouse first entered was scored as the preferred arm and the other arm discriminated. After each passage, the device was cleaned with alcohol (70% ethanol), in order to eliminate as much as possible the odorous traces left by the previous mouse. (Taïwe et al., 2011 ; Taiwe et al., 2015).

On the second day (acquisition phase), each animal was placed in a starting position (at the end of the starting arm) to explore the device for 10 min. However, the entrance to the opposite arm (new arm) was closed, while the entrance to the other arm (familiar arm) was open. The mice were returned to their cages.

On the next day (retention phase), the mice were reintroduced into the device for 5 min. During this phase, the entrance to both arms (familiar new) was opened. The number of visits and the time spent in these two arms were recorded. The number of visits and the time spent in the starting arm were also recorded using a stopwatch (Taiwe et al., 2015).

2.5.2. Elevated Plus Maze Test

The device used was a locally made wooden labyrinth. The height of the device from the ground was 50 cm. In height, the device consisted of two opposing open arms (16 cm × 5 cm), two opposing closed arms (16 cm × 5cm × 10 cm) and with a platform in the center of the device (5 cm × 5 cm). The elevated plus maze test is an exteroceptive behavioral model for assessing learning and memory in mice (Sankar et *al*., 2007). This test, which took place over two successive days, was carried out according to the method described by Taiwe et al., (2015) . For a duration of 60 s, each animal was placed at the end of one of the open arms and the learning latency (time taken to enter one of the closed arms) was recorded using a stopwatch. The mouse was recognized as being in a closed arm when it entered with all four legs in the closed arm. The mouse was then allowed to move freely for an additional 10 s in that arm. Twenty-four hours later, the retention latency, i.e., the time taken to enter one of the closed arms was determined. A time of 60 s was given to mice when they did not enter one of the closed arms.

2.5.3. Open field test

The open field test is commonly used to assess locomotor activity, exploration and emotional reactivity in rodents (Belzung, 1999). One hour after undergoing the object recognition test, each mouse was observed for a period of 5 minutes in the open field. The test consisted of placing the mice one after the other in the center of the device, in order to allow them free exploration. Several behavioral parameters were evaluated: the number of lines crossed, the number of times spent the animal cleaned its body, the number of sit-ups (when the animal stood up on its hind legs and leaned on the edges of the experimental device) and the time spent in the center of the experimental device. After 5 minutes of observation, the mouse was returned to its original cage and the experimental device was cleaned with ethyl alcohol (70%) before introducing the next mouse (Ngo Bum et al.*,* 2009).

**2.6. Estimation of biochemical parameters in the hippocampus**

2.6.1. Sacrifice, seahorse harvesting and preparation of homogenates

Immediately after the previous study, the animals were sacrificed by cervical dislocation, the brains were harvested, washed in 0.9% NaCl, wrung out on toilet paper, placed in boxes containing saline (0.9% NaCl) frozen for solidification for 1 hour. Then, the brains were dissected on a dissecting table kept cold to extract the hippocampus. A mass of 0.1 g of hippocampus from each animal was added to 1 mL of Tris-HCl buffer (50 mM; KCl 150 mM; pH 7.4). After grinding in a potter, the mixture was introduced into a labeled tube and centrifuged at 10,000 rpm for 15 minutes. The supernatant was then collected and stored at -20 °C for estimation of biochemical parameters.

2.6.2. Evaluation of gamma aminobutyric acid (GABA) concentration

The level of GABA was assessed based on the method of (Löscher, 1980). After euthanasia, hippocampi were rapidly removed, blotted, weighed, and put into ice-cold 5 ml trichloroacetic acid (TCA, 10%, w/v) with a glass homogenizer. The obtained mixture was homogenized and centrifuged at 10 000 rpm for 10 min at 0 ◦C. A sample (0.1 ml) of brain tissue extract was collected in 0.2 ml of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.9), heated in a water bath at 60 ◦C for 30 min, then cooled at room temperature (26-27 ◦C) and treated with 5 ml of freshly prepared alkaline copper tartrate reagent (0.16% disodium carbonate and 0.03% copper sulfate and 0.0329% tartaric acid), vortexed and incubated for 15 min at 25 ◦C. After 10 min of cooling at room temperature, the absorbance was read at 451 nm with a spectroﬂuorimeter. For GABA standards, different amounts (20, 40, 60, 80, and 100 μg) mixed with 1.5 μg glutamic acid were dissolved in 1 ml of 10% TCA (w/v). Gamma-aminobutyric acid was determined by the measurement of the formed ﬂuorescent product resulting from the reaction of GABA with ninhydrin in an alkaline medium in the presence of glutamate. The GABA content in the hippocampi was expressed in μg/g of wet tissue.

2.6.3. Evaluation of GABA-transaminase activity

The colorimetric method of determination of the GABA-T activity is based on the interaction of 3-methyl-2-benzothiazole-2-hydrazone (MBTH) with succinic semialdehyde (SSA) which appears during the enzymatic reaction (Sytinsky *et al*., 1991 ; Moto *et al*., 2018 ; Kandeda *et al*., 2021). Collected hippocampi brain tissues were then washed to remove blood, blotted to dry, then submerged in 5 ml of methanol (75%, v/v) and homogenized using a glass Teﬂon homogenizer for 2 min. The homogenate was centrifuged at 10 000 rpm at − 10 ◦C for 15 min (Nayak and Chatterjee, 2003), and the GABA-T activity in the hippocampi was measured spectrophotometrically as described by Sytinsky et al. (Sytinsky *et* al., 1991) with few modifcations (Nayak and Chatterjee, 2003). To a 10-ml volumetric ﬂask, 15 μmol from each of α-oxoglutarate and GABA, 10 μg of pyridoxal phosphate, and 1 ml of the supernatant of brain tissue (10% in sucrose, 0.32 mmol/l) were added, and the final volume was made up to 3 ml with buffer containing 0.2 M Tris-HCl (pH 8.6). The final mixture was incubated at 37 ◦C for 30 min for reaction. The reaction was terminated by the addition of 0.5 ml ice-cold 20% trichloroacetic acid (TCA). The blank was prepared by replacing the homogenate with methanol from the mixture. The succinic semialdehyde (SSA) produced in the incubation mixture was quantifed at 610 nm in spectroﬂuorimeter. The color complex of SSA and MBTH in the presence of 1 ml of 12% FeCl3 (pH 2), followed in 5 min by the addition of 2 ml of acetone (4.4%, v/v), was measured against the blank. The GABA-T activity was measured in pg/min/mg of protein.

2.6.4. Assessment of acetylcholinesterase activity

The AchE activity was assessed by the Ellman method (Ellman *et* al., 1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8, Sigma–Aldrich), 0.1 ml of acetylthiocholine iodide (Sigma–Aldrich) and 0.1 ml of DNTB (Ellman reagent, Sigma–Aldrich). The change in absorbance was measured at 412 nm for 2 min, at 30 s intervals. Results were expressed in U/min/mg of protein in the tissue (1 U/min/mg of AchE was defined as the amount of enzyme that hydrolyzed 1 mmol of acetylthiocholine iodide).

**2.7. Phytochemical analysis of *Crassocephalum bauchiense***

Phytochemical characterization tests on the aqueous extract of *Crassocephalum bauchiense* were carried out using the qualitative colorimetric methods of Harbone (1976) and taiwe et al., (2010) to determine the main chemical groups or families.

**2.8. Statistical Analysis**

Statistical analyses of the obtained values and construction of the graphs were performed using Graph Pad Prism version 8.0.1, Microsoft Office Excel 2013 software. The results were expressed as mean ± standard error on the mean (SEM) or as a percentage. The different values were compared using the analysis of variance test (One-way ANOVA) and, when differences existed, Tukey's multiple comparison test was used to highlight the significances between the different groups. Fisher's exact probability was used to compare the different percentages. At p<0.05, differences were considered significant.

**3. RESULTS**

**3.1. Phytochemical analysis of the aqueous extract of *Crassocephalum bauchiense***

The analysis of the phytochemical composition of the aqueous extract of the leafy stems of *Crassocephalum bauchiense* revealed the presence of alkaloids, flavonoids, catechotanins, phlobotanins, triterpenes, saponins and polyphenols.

**3.2. Effect of Crassocephalum bauchiense on status epilepticus latency induced by pilocarpine**

Significant inter-group differences were observed in the *status epilepticus*. Administration of pilocarpine induced seizure onset in the negative control (18.67 ± 0.87 min) compared to the normal control (0.00 ± 0.00 min). *Crassocephalum bauchiense* extract delayed latency[F (6, 35) = 259, *P* <0.001] seizure onset time by 34.81 ± 1.09 min and 38.59 ± 1.25 min at 140 and 280 mg/kg, respectively Similarly, sodium valproate significantly increased (p <0.001) this time to 41.04 ± 4.77 min compared with the negative control **(Fig.1).**



**Fig.1. Effect of *Crassocephalum bauchiense* on the *status epilepticus* latency of pilocarpine-induced seizures**.

*Each bar represents the mean ± MSE, n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test.; \*\*\*p < 0.001; significant difference compared to the negative control (DW+Pilo).* *normal control group (DW+DW); DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg).*

**3.****3. Effect of Crassocephalum bauchiense on latency and seizure score 24 hours after induction of SE with pilocarpine**

The administration of pilocarpine induced the onset of the first tonic seizure in the negative control (87.83 ± 4.95 s) compared to the normal control (0.00 ± 00.00). *Crassocephalum bauchiense* extract administered orally to mice delayed [ F (6, 35) = 1320, *P* <0.001] the onset of tonic-clonic convulsions by 662.5 ± 68.55 and 918.66 ± 48.61 s in the 140 and 280 mg/kg dose groups, respectively, compared to the negative control. The same was true for sodium valproate, which induced an increase (p<0.001) in the latency of onset of tonic-clonic seizures to 1587.83 ± 62.64 s compared to the negative control **(Fig.2A)**.

It was found that the seizure score increased (p <0.001) to 1 (100%) in the normal control which received distilled water compared to the negative control which received distilled water and pilocarpine in which this score was 0 (0%) **(Fig.2B)**. This score increased [ F (6, 35) = 1123, *P* <0.001] in a dose-dependent manner by 0.05 (5 %), 0.36 (36%), 0.86 (86%), 0.90 (90%) and respectively, in mice which received 28, 70, 140, and 280 mg/kg dose of extract of *Crassocephalum bauchiense* compared with the negative control. Sodium valproate increased (p <0.001) this score to 0.94 (94%) compared with the negative control **(Fig.2B)**.



**Fig. 2. Effect of *Crassocephalum bauchiense* on the latency of the first tonic-clonic seizure (A) and score of pilocarpine-induced seizures (B)**.

*Each bar represents the mean ± MSE, n = 6. The data were analyzed by the one-way ANOVA, followed by the turkey test.; \*\*\*p < 0.001; significant difference compared to the negative control (DW+Pilo). normal control group (DW+DW); DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg).*

**3.4. Effects of aqueous extract of Crassocephalum bauchiense on the number of tonic and clonic convulsions induced by pilocarpine**

The number of tonic convulsions increased significantly (p <0.001) in the negative control (7.67 ± 1.21) compared with the normal control (0.00 ± 0.00). *Crassocephalum bauchiense* extract induced a significant decrease [F (6, 35) = 50.5, *P* <0.001] of this number by 5.5 (p <0.05) (28.19%), 4.33 (p <0.001) (43.86%) and 4.33 (p <0.001) (43.66%) respectively in mice given the dose of 70, 140 and 280 mg/kg compared to the negative control **(Table 1)**. As for sodium valproate, it induced a decrease (p <0.001) of 2.67 (69.58%) in the number of tonic convulsions compared to the negative control **(Table 1)**.

The number of clonic convulsions was significantly increased (p <0.001) in the negative control (24.33 ± 2.06) compared with the normal control (0.00 ± 0.00). The aqueous extract of *Crassocephalum bauchiense* significantly [F (6, 35) = 135, *P* <0.001] decreased the number of clonic convulsions in mice. This decrease was from 12.33 (49.32%) to 10.00 (58.89%) respectively in the 140 and 280 mg/kg mice compared to the negative control **(Table 1)**. The same is true for sodium valproate, which induced a decrease (p<0.001) of 3.16 (87.01%) in clonic convulsions compared to the negative control **(Table 1)**.

**Table 1: Number of tonic and clonic convulsions according to the doses of** ***Crassocephalum bauchiense***

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **Doses (mg/kg)** | **Number of tonic seizures** | **Number of clonic seizures** |
| **DW+DW** | - | 0.00 ± 0.00 | 0.00 ± 0.00 |
| **DW+Pilo** | - | 7.66 ± 1.21£££ | 24.33 ± 2.06 £££ |
| **Cb28+Pilo** | 28 | 7.83 ± 1.16 £££ | 22.66 ± 2.50 £££ |
| **Cb70+Pilo** | 70 | 5.50 ± 1.04 £££ \* | 19.66 ± 3.20 £££ \*\* |
| **Cb140+Pilo** | 140 | 4.33 ± 1.03 £££ \*\*\* | 12.33 ± 2.03 £££ \*\*\* |
| **Cb280+Pilo** | 280 | 4.33 ± 1.03 £££ \*\*\* | 10.00 ± 1.41 £££ \*\*\* |
| **SV+Pilo** | 300 | 2.66 ± 0.51 £££ \*\*\* | 3.16 ± 0.98 \*\*\* |

*Results are expressed as means ± S.E.M n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; significant difference compared to the negative control (DW+Pilo).* ***£££****p<0.001; significant difference compared to the normal control* *(DW+DW); DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg).*

**3.5. Effects of aqueous extract of *Crassocephalum bauchiense* on the duration of tonic and clonic convulsions induced by pilocarpine**

. The duration of tonic convulsion increased (p <0.001) in the negative control (14.66 ± 0.81 s) in contrast to the normal control (0.00 ± 0.00 s). Aqueous extract of *Crassocephalum bauchiense* induced a decrease [F (6, 35) = 188, *P* <0.001] of 6.33 ± 1.03 s (56.82%) and 5.83 ± 1.16 s (60.23%) respectively in mice dosed with 140 and 280 mg/kg compared to the negative control **(Table 2)**. Similarly, sodium valproate decreased (p <0.001) this time to 4.33 ± 1.36 s (70.46%) compared to the negative control **(Table 2)**.

The duration of clonic convulsion was significantly increased (p <0.001) in the negative control (42.83 ± 5.41 sec) compared to the normal control (0.00 ± 0.00 s). Aqueous extract of *Crassocephalum bauchiense* induced a decrease [F (6, 35) = 133, *P* <0.001] from 13.83 ± 1.16 (67.70%) to 12.66 ± 1.75 (70.44%) in the duration of clonic convulsions respectively in mice given 140 and 280 mg/kg compared to the negative control **(Table 2)**. Similarly, sodium valproate led to a decrease of 6.66 ± 2.06 s (84.45%) compared to the negative control **(Table 2)**.

**Table 2: Duration of tonic and clonic convulsions as a function** **of *Crassocephalum bauchiense* doses**

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **Doses (mg/kg)** | **Duration of tonic seizures (s)** | **Duration of clonic seizures (s)** |
| **DW+DW** | - | 0.00 ± 0.00 | 0.00 ± 0.00 |
| **DW+Pilo** | - | 14.66 ± 0.81£££ | 42.83 ± 5.41£££ |
| **Cb28+Pilo** | 28 | 14.16 ± 0.75£££ | 31.16 ± 2.87£££ \*\*\* |
| **Cb70+Pilo** | 70 | 13.66 ± 1.03£££ | 29.66 ± 5.31£££ \*\*\* |
| **Cb140+Pilo** | 140 | 6.33 ± 1.03£££ \*\*\* | 13.83 ± 1.16£££ \*\*\* |
| **Cb280+Pilo** | 280 | 5.83 ± 1.16£££ \*\*\* | 12.66 ± 1.75£££ \*\*\* |
| **SV+Pilo** | 300 | 4.33 ± 1.36£££ \*\*\* | 6.66 ± 2.06£ \*\*\* |

*Results are expressed as means ± S.E.M n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*\*\*p < 0.001 ; significant difference compared to the negative control (DW+Pilo). £p<0.05 ; £££p<0.001 ; significant difference compared to the normal control (DW+DW) ; DW:* *Distilled Water (10 mL/kg) ; Pilo: Pilocarpine; Cb: Crassocephalum bauchiense; SV: Sodium Valproate (300 mg/kg).*

**3.6.** **Anti-amnesic effects of aqueous extract of Crassocephalum bauchiense in epileptic mice subjected to the T-maze test**

The Latency to choose the preferred arm increased (p <0.001) in the negative control (86.5 ± 5.83 s) compared to the normal control (21.16 ± 4.5 s) **(Fig.3A)**. In mice treated with aqueous extract of *C. bauchiense*, the latency time of choosing an arm of the T-maze decreased [ F (7, 40) = 207, P<0.001] by 69.5 ± 4.33 s, 24.16 ± 3.5 s and 17.16 ± 2.5 s respectively in mice that received the doses of 70, 140 and 280 mg/kg **(Fig.3A)**. Sodium valproate and piracetam also decreased this time (p <0.001) 17.33 ± 2.78 s and 15.5 ± 2.16 s respectively compared to the negative control **(Fig.3A)**.

The number of returns in the baseline arm increased (p <0.001) in the negative control (22.33 ± 1.67) compared to the normal control (14.67 ± 1.78) **(Fig.3B)**. Mice treated with aqueous extract of *Crassocephalum bauchiense* decreased [F (7, 40) = 13, P<0.001] this number, 17.83 ± 1.89 (p <0.05), 15.83 ± 2.16 (p <0.001), 14.67 ± 2.56 (p <0.001), 11.5 ± 1.33 (p <0.001) respectively in mice that received the doses of 28, 70, 140 and 280 mg/kg **(Fig.3B)**. Sodium valproate and piracetam also decreased (p <0.001) this number to 11.83 ± 1.27 and 12.33 ± 1.22, respectively, compared with the negative control **(Fig.3B)**.

The number of entries into the preferred arm decreased (p <0.001) in the negative control (4.5 ± 1.5) compared to the control at normal (12.83 ± 3.11) **(Fig.3C)**. Mice treated with aqueous extract of *Crassocephalum bauchiense* increased [F (7, 40) = 6,59, *P*<0.001] this number, 11.83 ± 2.17 (p <0.05), 14.67 ± 3.89 (p <0.01), 15.17 ± 3.56 (p <0.001) respectively in mice that received the doses of 70, 140 and 280 mg/kg **(Fig.3C)**. Sodium valproate and piracetam increased this number to 14.16 ± 4.17 (p <0.01) and 16.83 ± 2.83 (p <0.001), respectively, compared with the negative control **(Fig.3C)**.

In the negative control, the number of entries (13.16 ± 1.5) in the discriminated arm increased (p <0.01) compared to the control at normal (6.83 ± 1.16) **(Fig.3D)**. Mice treated with aqueous extract of *Crassocephalum bauchiense* decreased [F (7, 40) = 5,81, *P*<0.001] this number, 8.16 ± 1.22 (p <0.05), 8.5 ± 2.17 (p <0.05), 6.5 ± 2.83 (p <0.001), respectively in mice that received the doses of 70, 140 and 280 mg/kg **(Fig.3D)**. Sodium valproate and piracetam also decreased this number (p <0.001) to 6.83 ± 2.16 and 6.16 ± 1.83, respectively, compared with the negative control **(Fig.3D)**.

The time spent in the preferred arm of the maze decreased (p <0.001) in the negative control (28.5 ± 4.71 s) compared to the normal control (71.83 ± 1.85 s) **(Fig.3E).** In mice treated with aqueous extract of *C. bauchiense*, the time spent in the preferred arm of the T-maze decreased [F (7, 40) = 108, *P*<0.001] by 52.17 ± 4.14 s (p <0.01), 65.17 ± 1.9 s (p <0.001), 95.83 ± 5.33 s (p <0.001), 121.83 ± 8.67 s (p <0.001), respectively, in mice that received the 70, 140, and 280 mg/kg doses **(Fig.3E)**. Sodium valproate and piracetam also decreased this time (p <0.001) 117.33 ± 5.23 s (p <0.001) and 138.83 ±9.85 sec (p <0.001), respectively, compared to the negative control **(Fig.3E)**.

The time spent in the discriminated arm increased (p <0.001) in the negative control (64.83 ± 4.5 s) compared to the normal control which has a time (23.83 ± 3.88 s) **(Fig.3F)**. In mice treated with aqueous extract of *C. bauchiense*, the time spent in the discriminated arm decreased [F (7, 40) = 31,5, *P*<0.001]. by 50.33 ± 8.33 s (p <0.05), 37.17 ± 7.56 s (p <0.001), 38.16 ± 2.27 s (p <0.001), 18.17 ± 4.83 s (p <0.001) respectively in mice that received the doses of 28, 70, 140 and 280 mg/kg **(Fig.3F)**. Sodium valproate and imipramine also decreased this time (p <0.001) 23.33 ± 2.67 s (p <0.001) and 25.33 ± 4.67 s (p <0.001), respectively, compared with the negative control **(Fig.3F)**.



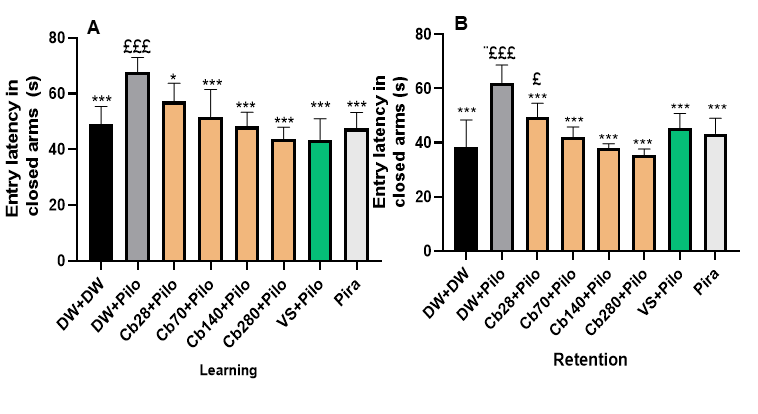
**Fig.3. Effect of *Crassocephalum bauchiense* on the Latency to choose the preferred arm (A), the return number in starting arm (B), the number entries in the preferred arm (C), the number of entries in the discriminated arm (D), the time spent in the preferred arm (E) and the time spent in the discriminated arm (F)**.

*Each bar represents the mean ± ESM, n = 6. The data were analyzed by the one-way ANOVA, followed by the tukey test. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; significant difference compared to the negative control (DW+DW).* ***£****p<0.05;* ***££****p<0.01;* ***£££****p<0.001; significant difference compared to the normal control (DW+DW); DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg).*

**3.7. Anti-amnestic effects of aqueous extract of Crassocephalum bauchiense in epileptic mice subjected to the elevated cross maze test**

During the learning phase, significant inter-group differences were observed in the entry latency into one of the closed arms the entry latency into one of the closed arms increased (p <0.001) in the negative control (67.83 ± 4.11 s) compared to the normal control (49.17 ± 4.5 s) **(Fig.4A)**. In mice treated with aqueous extract of *C. bauchiense*, the latency of entry into one of the closed arms decreased [F (7, 40) = 9,27, *P*<0.001] by 57.17 ± 4.83 s (p <0.05), 51.67 ± 8.33 s (p <0.001), 48.17 ± 3.83 s (p <0.001), 43.67 ± 2.89 s (p <0.001) in mice that received the 28, 70, 140, and 280 mg/kg doses, respectively **(Fig.4A)**. Sodium valproate and imipramine also decreased this time (p <0.001) to 47.5 ± 4.83 s (p <0.001) and 42.17 ± 2.28 s (p <0.001), respectively, compared with the negative control **(Fig.4A)**.

During the retention phase, significant inter-group differences were observed in the entry latency into one of the closed arms. the entry latency into one of the closed arms increased (p <0.001) in the negative control (62.17 ± 4.83 s) compared to the normal control (38.5 ± 7.17 s) **(Fig.4B)**. In mice treated with aqueous extract of *C. bauchiense*, the latency of entry into one of the closed arms decreased [F (7, 40) = 14,0, *P*<0.001] by 49.5 ± 3.83 s (p <0.01), 42.17 ± 2.28 s (p <0.001), 38.83 ± 2.16 s (p <0.001), 35.33 ± 1.67 s (p <0.001) in mice that received the 28, 70, 140, and 280 mg/kg doses, respectively **(Fig.4B)**. Sodium valproate and imipramine also decreased this time (p <0.001) 45.5 ± 4.33 s (p <0.001) and 40.67 ± 3.11 s (p <0.001), respectively, compared with the negative control **(Fig.4B)**.



**Fig.4. Effect of *Crassocephalum bauchiense* on the entry latency in closed arms.**

*Each bar represents the mean ± ESM, n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; significant difference compared to the negative control (DW+Pilo).* ***£****p<0.05;* ***££****p<0.01;* ***£££****p<0.001; significant difference compared to the normal control (DW+DW);* *DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg); Pira: Piracetam (15 mg/kg).*

**3.8. Effect of aqueous extract of Crassocephalum bauchiense on exploration behavior in mice in the open field**

The number of crossed lines decreased (p <0.05) in the negative control (6.50 ± 1.17) compared to the normal control (10.33 ± 1.11) **(Table 3)**. We observe an increase [F (7, 40) = 77,7, *P*<0.001] in the number of crossed lines of 16.83 ± 0.89 (p <0.01), 23.33 ± 2.11 (p <0.001), 32.17 ± 3.22 (p <0.001) and 33.17 ± 1.17 (p <0.001) respectively in the mice given the different doses of 28, 70, 140 and 280 mg/kg compared to the negative control **(Table 3)**. The number of lines crossed was significantly increased in mice treated with sodium valproate, 37.67 ± 1.67 (p <0.001) and piracetam, 39.83 ± 5.89 (p <0.001) **(Table 3)**.

The number of rearing increased (p <0.001) in the negative control (11.50 ± 1.17) compared to the normal control (8.17 ± 1.55) **(Table 3)**. The extract significantly (p <0.001) decreased [F (7, 40) = 72,3, *P*<0.001] the number of turnarounds by 2.83 ± 0.55, 2.83 ± 0.83, 1.83 ± 0.55 and 1.17 ± 0.28 respectively in the mice that received the different doses of 28, 70, 140 and 280 mg/kg of the plant **(Table 3)**. Sodium valproate and piracetam significantly decreased the number of crossed lines to 1.33 ± 0.44 (p <0.001) to 1.50 ± 0.50 (p <0.001) respectively **(Table 3)**.

The number of times the animal cleans its body (grooming) decreased in the negative control (1.00 ± 0.33) compared to the normal control (2.50 ± 0.83) **(Table 3)**. This number increased [F (7, 40) = 5,22, *P*<0.001] significantly from 2.83 ± 0.55 (p <0.05) to 3.50 ± 0.83 (p <0.001) in the groups of animals treated with 140 and 280 mg/kg of *Crassocephalum bauchiense* extract, respectively **(Table 3)**. Sodium valproate and piracetam also increased the number of "grooming" to 3.67 ± 0.78 (p <0.001) and 2.83 ± 0.83 (p <0.05) respectively **(Table 3)**.

The time spent in the center of the open field was significantly decreased in the negative control (2.50 ± 0.50 s) compared to the normal control (4.83 ± 0.89 s). **(Table 3)**. Mice treated with the different doses of *Crassocephalum bauchiense* showed a significant increase [F (7, 40) = 94,2, *P*<0.001]. in time spent in the center of, 18.16 ± 1.22 sec (p <0.001) and 21.16 ± 0.83 s (p <0.001) respectively in those treated with the140 and 280 mg/kg doses of the extract. The time spent in the center increased in mice treated with sodium valproate and piracetam to 24.67 ± 1.67 s (p <0.001) and 30.67 ± 5.22 s (p <0.001), respectively **(Table 3)**.

**Table 3**: **Effects of *Crassocephalum bauchiense* on the open field**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Doses (mg/kg)** | **Number of "rearing"** | **Number of "crossing"** | **Number of "grooming** | **Time spent in the center (s)** |
| DW+DW | - | 8.16 ± 1.15 | 10,33 ± 1.11 | 2.50 ± 0.83 | 4.83 ± 0.89 |
| DW+Pilo | - | 11.50 ± 1.67 £££ | 6.50 ± 1.17 | 1.00 ± 0.33 | 2.50 ± 0.5 £ |
| Cb28+Pilo | 28 | 2.83 ± 0.55 £££\*\*\* | 16,83 ± 0.89 \*\*\* | 2.67 ± 0.44\* | 4.50 ± 0.83 |
| Cb70+Pilo | 70 | 1.83 ± 0.83 £££\*\*\* | 23.33 ± 2.11 £££\*\*\* | 2.33 ± 0.66 | 11.50 ± 1.16 ££\*\*\* |
| Cb140+Pilo | 140 | 1.83 ± 0.55 £££\*\*\* | 32.16 ± 3,22 £££\*\*\* | 4.83 ± 0.56 \* | 18.17 ± 1.22 £££\*\*\* |
| Cb280+Pilo | 280 | 1.83 ± 0.55 £££\*\*\* | 33.16 ± 1.17 £££\*\*\* | 3.50 ± 0.83 \*\*\* | 21.17 ± 0.83 £££\*\*\* |
| SV+Pilo | 300 | 1.16 ± 0.28 £££\*\*\* | 37.67 ± 1.67 £££\*\*\* | 3.67 ± 0.78 \*\*\* | 24.67 ± 1.67 £££\*\*\* |
| Pira | 200 | 1.50 ± 0.50 £££\*\*\* | 39.83 ± 5.89 £££\*\*\* | 2.83 ± 0.83 \*\*\* | 30.66 ± 5.22 £££\*\*\* |

*Results are expressed as means ± S.E.M n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*p < 0.05 ; \*\*\*p < 0.001; significant difference compared to the negative control (DW+DW).* ***£****p<0.05;* ***££****p<0.01;* ***£££****p<0.001; significant difference compared to the normal control (DW+DW).* *DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg); Pira: Piracetam (15 mg/kg).*

**3.9. Effect of aqueous extract of *Crassocephalum bauchiense* on GABA metabolism in the hippocampus**

The GABA concentration reveals a significant decrease (p <0.001) in the negative control (263.5 ± 9.33 μg/g tissue) compared to the normal control (392.16 μg/g tissue) **(Table 4)**. The aqueous extract of *Crassocephalum bauchiense* led to an increase [(F (7, 40) = 223, *P*<0.001] in GABA concentration in mice in the test groups, 302.16 ± 1.88 µg/g (p <0.001), 379.16 ± 7.83 µg/g (p <0.001) and 387.66 ± 8.77 µg/g (p <0.001) respectively in those treated with the 70, 140 and 280 mg/kg doses **(Table 4)**. The same is true for sodium valproate (300 mg/kg) and piracetam induced a significant increase in GABA level 387.33 ± 5.11 μg/g tissue (p <0.001) and 389.50 ± 6.83 μg/g (p <0.001), respectively, compared with the negative control **(Table 4)**.

There was a significant increase in GABA-transaminase activity, in mice of the negative control group (115.66 ± 8.33 pg/min/mg of tissue) compared to the normal control (44.16 pg/min/mg of tissue) **(Table 4)**. *Crassocephalum bauchiense* extract led to a decrease [F (7, 40) = 146, *P*<0.001]. In this concentration of 95.50 ± 2.00 pg/min/mg (p <0.001), 81.16 ± 4.50 pg/min/mg (p <0.001), 49.66 ± 4.00 pg/min/mg (p <0.001), and 41.33 ± 2.11 pg/min/mg (p <0.001) respectively in mice treated with 28, 70, 140, and 280 mg/kg **(Table 4)**. Sodium valproate and piracetam significantly decreased this activity to 42.16 ± 2.28 pg/min/mg tissue (p <0.001) and 41.50 ± 3.66 pg/min/mg tissue (p <0.001), respectively, compared with the negative control **(Table 4)**.

**Table 4:Effect of *Crassocephalum bauchiense* on the GABA metabolism in the hippocampu**s

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **Doses (mg/kg)** | **GABA**  **(μg/g de tissu)** | **GABA-Transaminase (pg/min/mg de tissu)** |
| DW+DW | -- | 392.16 ± 2.83 | 44.16 ± 6.16 |
| DW+Pilo | -- | 263.50 ± 9.33 £££ | 115.66 ± 8.33 £££ |
| Cb28+Pilo | 28 | 258.16 ± 8.61 £££ | 95.50 ± 2.00 £££ \*\*\* |
| Cb70+Pilo | 70 | 302.16 ± 1.88 £££ \*\*\* | 81.16 ± 4.50 £££ \*\*\* |
| Cb140+Pilo | 140 | 379.16 ± 7.83 \*\*\* | 49.66 ± 4.00 \*\*\* |
| Cb280+Pilo | 280 | 387.66 ± 8.77 \*\*\* | 41.33 ± 2.11 \*\*\* |
| SV+Pilo | 300 | 387.33 ± 5.11 \*\*\* | 42.16 ± 2.27 \*\*\* |
| Pira | 200 | 389.50 ± 6.83 \*\*\* | 41.50 ± 3.66 \*\*\* |

*Results are expressed as means ± S.E.M n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; significant difference compared to the negative control (DW+DW).* ***£****p<0.05;* ***££****p<0.01;* ***£££****p<0.001; significant difference compared to the normal control (DW+Pilo). DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg); Pira: Piracetam (15 mg/kg).*

**3.10. Effect of aqueous extract of *Crassocephalum bauchiense* on hippocampal AchE activity**

The hippocampal AchE activity increased (p <0.001) in mice of the negative control group (14.58 ± 0.44 µmol/min/mg tissue) compared to the normal control (10.34 ± 0.58 µmol/min/mg tissue) **(Fig.5)**. *Crassocephalum bauchiense* extract led to a decrease [F (7, 40) = 14,9 *P*<0.001]. in this activity of 12.01 ± 1.03 µmol/min/mg of tissue, 11.78 ± 0.63 µmol/min/mg of tissue, 10.81 ± 0.95 µmol/min/mg of tissue, and 10.67 ± 0.59 µmol/min/mg of tissue respectively in mice treated with 28, 70, 140 and 280 mg/kg **(Fig.5)**. Sodium valproate and piracetam significantly (p<0.001) decreased this activity, respectively 9.92 ± 0.39 µmol/min/mg tissue and 10.70 ± 0.61 µmol/min/mg tissue compared to the negative control **(Fig.5)**.



**Fig.5. Effect of *Crassocephalum bauchiense* on the AchE activity**

*Each bar represents the mean ± ESM, n = 6. The data were analyzed by the one-way ANOVA, followed by the Tukey test. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; significant difference compared to the negative control (DW+Pilo).* ***£****p<0.05;* ***££****p<0.01;* ***£££****p<0.001; significant difference compared to the normal control (DW+DW). DW: Distilled Water (10 mL/kg); Pilo: Pilocarpine; Cb: C. bauchiense; SV: Sodium Valproate (300 mg/kg); Pira: Piracetam (15 mg/kg).*

**4. DISCUSSION**

“The objective of the present study was to evaluate the effects of a decoction of leafy stems of *Crassocephalum bauchiense* on seizures, learning and memory during pilocarpine-induced epileptogenesis in mice. The initial *status epilepticus* induced by pilocarpine is characterized by tonic-clonic seizures, which leads to a generalized and severe loss of neuronal cells in the hippocampus” (Mazzuferi et al., 2012). “Indeed, during status epilepticus, pilocarpine acts on the muscarinic M1 receptor by activating phospholipase C which produces diacylglycerol and inositol triphosphate which will allow the entry of Ca2+ ions into the cell and the increase of the brain excitability” (Segal, 1988). “The high concentration of Ca2+ favors the release of glutamate inducing the status epilepticus with consequent activation of lipases, proteases and nucleases that will cause the death of nerve cells by necrosis or apoptosis” (Scorza et al., 2009). “Pretreatment with clonazepam, phenobarbital, or sodium valproate has been shown to prevent limbic seizures and to protect against SE-related cellular damage. In the present study, pretreatment of mice with the aqueous extract of *Crassocephalum bauchiense* (140 and 280 mg/kg) significantly protected them from the effects of SE. The results show that *Crassocephalum bauchiense* significantly decreased the onset of seizures by increasing SE latency, first tonic-clonic seizure latency and seizure score. Such a reduction in seizure severity and sensitivity to a convulsant suggests that the extract antagonizes or modifies the epileptogenic process induced by pilocarpine” (Pitkänen et al., 2005; Kandeda et al., 2017). “The effects of *Crassocephalum bauchiense* at high doses (280 mg/kg) are similar to those of sodium valproate, a reference antiepileptic drug. The primary mechanisms of sodium valproate include an increase in gabaergic activity, a reduction in excitatory neurotransmission, and a modification of monoamines” (Xavier et *al*., 2007 ; Rahmati et *al*., 2013 ; Taiwe et al., 2015). “These observations suggest that *Crassocephalum bauchiense* extract may have modified the epileptogenic process by increasing GABAergic activity and reducing excitatory neurotransmission. Phytochemical screening of *Crassocephalum bauchiense* extract revealed the presence of chemical compounds such as alkaloids, flavonoids, saponins, polyphenols, triterpenes and tannins which would possess pharmacological properties. Flavonoids and alkaloids are compounds that act on the nervous system as anticonvulsants” (Massiot, 1994). “Saponins and flavonoids have been shown to inhibit epileptic seizures by antagonizing glutamatergic receptors or potentiating the GABAA receptor complex in vivo and in vitro models of seizures” (Penda et al., 2024).Such substances have been shown to have anticonvulsant and antiepileptic properties in several animal models of epilepsy (Ngo Bum et al., 2009) and particularly temporal lobe epilepsy  (Taiwe et al., 2015).

“During epileptogenesis, there is a decrease in the GABAergic inhibitory signal to the detriment of the glutamatergic excitatory signal. Pilocarpine increases GABA transaminase activity and decreases GABA levels in the brain as well as GABA receptor density in the striatum, frontal cortex, and hippocampus” (Abel et al., 1999). “The significant decrease in GABA concentration in the hippocampus of epileptic mice that received distilled water suggests a greater use of this amino acid in an attempt to counteract the hyperexcitability of surrounding tissues. This decrease in GABA levels could also be explained by the increase in GABA-T activity during this phase” (Cavalheiro et al., 2006). “Our results show a significant increase in GABA levels in the hippocampus of mice from test batches. Thus, the aqueous extract of *Crassocephalum bauchiense* showed increasing concentrations of GABA and a significant decrease in GABA-T. However, since GABA cannot cross the blood-brain barrier, the increase in cerebral GABA would be due to the presence in the extract of certain compounds that can interact with GABAergic neurotransmission” (Ngo Bum et al., 2001). “Indeed, saponins, alkaloids and flavonoids are known to increase the activity of the GABAergic system through different interactions including potentiation of the GABA receptor complex, increase in GABA concentration” (Singab et al., 2015). “In addition, flavonoids act on the GABAA complex as the alkaloids interact with the voltage-gated Na+ channel causing a blockage, responsible for reducing neuronal excitability” (Zhu et al., 2014; Ngaibi et al., 2024). “Saponins and phenolic compounds have an inhibitory action on different Ca+ channels, reducing their opening times and prolonging their closing times” (Zhong et *al*., 1995; Kim et al., 2008). “*Crasocephalum bauchiense* seems to produce its anticonvulsant effects by enhancing gabaergic neurotransmission through the benzodiazepine receptor site. Its action on the benzodiazepine receptor site would increase the entry of chloride ions into the postsynaptic membrane, which would increase brain inhibition, by increasing the frequency of opening of ion channels in response to GABA”(Bacon and Viennot, 1990 ; Ngo Bum et al., 2001 ; Taïwe et al., 2011).

Since cognitive impairment or decline may also be associated with epileptogenesis in temporal lobe epilepsy (Cha et al., 2002 ; Kumar et al., 2008 ; Mehla et al., 2010). It has been shown that the animal model of pilocarpine-induced temporal lobe epilepsy can promote the change in AchE activity (Furtado et al.*,* 2002; De Sales Santos et al., 2010). Thus, in the damaged parts of the brains of mice that received a neurotoxic substance, there is a significant loss of cholinergic neurons associated with low concentrations of acetylcholine in the hippocampus (Zhou et al., 2007). This deficit results in a decrease in cognitive functions. The aqueous of extract *Crassocephalum bauchiense* improved the learning and memory process in mice in the T-maze test. Mice given a decoction of *Crassocephalum bauchiense* showed both, a decrease in the latency time to choose the preferred arm, a decrease in the time spent in the discriminated arm and a decrease in the number of returns to the starting arm on the one hand and on the other hand an increase in the number of entries and the time spent in the preferred arm, an increase in the number of entries in the discriminated arm. This decrease in latency to retrieve food suggests an improvement in reference memory (Taah Yamndou et al., 2021). These results indicate an increase in cognitive performance (Koto-te-Nyiwa et al., 2014). The elevated plus maze test and the open field test allowed us to reassure ourselves that the potential deficits observed during cognitive tests are not related to dysfunctions in sensorimotor abilities that could affect the navigation of mice (Schmitt, 2018). Neuron loss in the hippocampus is the first event characterizing epileptogenesis (Kandeda et al., 2017). Aqueous extract of *Crassocephalum bauchiense* significantly decreased the activity of AchE, a marker of neuronal loss (Duysen et al., 2002 ; Freitas et al., 2005). Reduced AchE activity leads to increased acetylcholine levels and improved cholinergic transmission (Cibelle de Melo Bastos et al., 2024). These results suggest that the extract has anti-amnesic properties and acts on epileptogenesis.

**5. CONCLUSION**

In summary, we evaluated the anticonvulsant and anti-amnesic effects of the aqueous extract of *Crassocephalum bauchiense* on a pilocarpine-induced temporal lobe epilepsy model in mice. Oral pretreatment with the extract resulted in a reduction in seizure severity and an improvement in cognitive function in mice. Overall and taking into account the activity of AchE, these results indicate that the aqueous extract of *Crassocephalum bauchiense* has anticonvulsant and anti-amnesic effects. Considering the therapeutic potential of *Crassocephalum bauchiense* extract through this study, future experiments to evaluate the antiepileptogenic effects will be developed. The beneficial effects of this extract observed in the present study suggest that *Crassocephalum bauchiense* could also be used to prevent epilepsy, especially temporal lobe epilepsy and cognitive disorders.

**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).

**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.

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