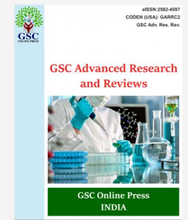
GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/



(Research Article)

Effects of *Phragmanthera capitata* (Loranthaceae) on a model of anxiety-like behaviours induced by chronic immobilization stress in mice

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GSC Advanced Research and Reviews, 2023, 17(02), 129–143

Publication history: Received on 24 September 2023; revised on 11 November 2023; accepted on 14 November 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.17.2.0420

Abstract

This work aims to evaluate the anxiolytic-like effects of *Phragmanthera capitata* in a model of chronic immobilization stress in mice. Groups of mice were treated in ten consecutive days as follows: a normal control group (NaCl 0.9% per os), a negative control group (chronic immobilization stress + NaCl 0.9% per os), three test groups that were submitted to chronic immobilization stress (CIS) and received three doses of the plant (25, 125, and 250 mg/kg, p.o), and a positive control group (chronic immobilization stress + diazepam 2 mg/kg, i.p). Open field and dark/light tests were used for the evaluation of anxiolytic effects. Antioxidant activities and the involvement of gabaergic neurotransmission were determined by measuring the levels of malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), gamma amino butyric acid (GABA), and GABA-transaminase (GABA-T) in the brain. Our results show that the highest dose of *Phragmanthera capitata* induced a significant increase ($p < 0.001$) of time spent in the centre, number of crossing and number of grooming in the open field test and a significant increase ($p < 0.001$) of time spent in the light compartment and the latency of the first escape in the light compartment of the dak/light test. The level of MDA and the activity of GABA-T were significantly decreased by the *Phragmanthera capitata* while reduced GSH, CAT and GABA, levels were increased. These results suggest that *Phragmanthera capitata* possesses anxiolytic-like effects that may be supported by its antioxidant activities and or the GABA neurotransmission.

Keywords: *Phragmanthera capitata;* Chronic; Immobilization; Stress; Anxiety

1. Introduction

Homeostasis is an essential phenomenon to life and well-being. It is an equilibrium state characterized by the steadiness of the inner environment of the body. This steadiness is frequently challenged by psychological and / or physiological stringency. The word “stress” is defined as an imbalanced homeostasis [1], [2] associated with behavioural, biochemical and physiological changes [3], [4]. It activates some physiologic and behavioural responses that act to reestablish and / or to maintain the homeostasis. The hippocampus and the amygdala are essential components of the neural circuitry mediating stress response. Inadequate, excessive and or prolonged stress response may lead to diseases. Several psychiatric disorders including anxiety are associated with inadequate GABA brain activity or low levels of GABA and high free radicals production. Stress response and anxiety share some neural circuitry mediating components supporting the involvement of stress in mood disorders. Anxiety can occur when a combination of internal and or external stresses exceeds the normal stress response or when the normal coping ability of a person is reduced for any reason. Anxiety is a generalized mood condition that can occur without an identifiable stressor [5], [6]; it is a mental

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state of turmoil and restlessness with an indefinable feeling of insecurity and objectless fear. Anxiety disorders are currently among the ten most important public health concerns, according to the World Health Organization and have reached epidemic proportion [7]. In Cameroon, anxiety disorders are among the top three mental diseases [8]. These disorders are recognized as main risk factors for many other diseases, including cardiovascular, metabolic and neuropsychiatric diseases [9], [7]. Moreover, anxiety is among the most prevalent mental disorder with very high comorbidity and severe impact on quality of life [10]. Anxiety disorders have prevalence about 21% over lifetime. They are manifested by spasms, palpitations, sweating, sweaty hands, dry mouth, dizziness and chest tightness [11]. Severe and or chronic stress has detrimental effects on physiologic functions including behaviours. Chronic immobilization stress is known to increase anxiety in mice; this phenomenon can be thwarted by drugs endowed of pharmacological activities such as neuroprotective, antioxidant, anti-depressant, and or anxiolytic activity [4]. Benzodiazepines are the most common drugs used for the treatment of anxiety. Because of the stigma of mental diseases and the side effects of anxiolytic drugs, patients mostly go to traditional healers in Africa [12]. It is becoming very useful to look for alternative and low cost effective herbal therapies especially in low income countries where the majority of the population relies on herbs remedies [13],[11]. A growing number of herbal medicines are being introduced into psychiatric practice on the basis of their efficacy and low side effects for the treatment of psychiatric disorders as severe depression and anxiety [14]. *Phragmanthera capitata* (Loranthaceae) is one of the medicinal plants used in Cameroon for the treatment of several diseases as nerve pain [15]. The mistletoe plant, *Phragmanthera capitata* (*P. capitata*) belongs to loranthaceae family and is widespread in Africa especially in Cameroon, Nigeria, Gabon and Côte d'Ivoire [16]. Cash crops such as avocado, cocoa, coffee, citrus appear strongly parasitized *by Phragmanthera capitata* [17]. This plant is known under several names according to the localities in Cameroon: Lihok (Bassa), Kolo'o me tobo (Ewondo), Torikoué (Bakoko) [15]. Phytochemical studies of Takem and colleagues revealed that *Phragmanthera capitata* harvested from avocado tree possess high presence of terpenoids and tannins; moderate presence of saponins, glycosides, anthraquinones and flavonoids; and low presence of alkaloids and phenols. Earlier scientific studies showed that *Phragmanthera capitata* has anti-diarrheal properties [18]; anti-pyretic and analgesic potentials [19]; and anxiety lowering potentials in a model of acute anxiety [20]. Nevertheless, this is the first study evaluating the anxiolytic-like effects of this plant on a chronic model of anxiety. The present work was design to assess the effects of *Phragmanthera capitata* on anxiety-like behaviour induced by chronic immobilization stress in mice.

2. Material and methods

2.1. Plant material

Leaves of *Phragmanthera capitata* were collected on *Persea americana* (avocado tree) from the centre region of Cameroon (Yaounde). A voucher specimen was deposited at the National Herbarium of Cameroon in Yaounde and identified under the number 24673/SRF/CAM

2.2. Preparation of the aqueous extract

Fresh leaves of *Phragmanthera capitata* were dried in the shade, ground and sieved. 3 grams of leaves powder were macerated in 24 ml of distilled water for 24 h and filtered with Whatman N°1 filter paper. 16.2 ml of the aqueous extract were obtained and were diluted (1/2 and 1/4) by adding distilled water. The filtrate (16.2 ml) was dried in an oven at 45 °C and 405 mg of brown solid was obtained with a yield of 13.5% (w/w). The following doses were used: 25,

2.3. Animals

Adult mice, *Mus musculus Swiss* (23 - 29 g), were obtained from the animal room of the Department of Biology and animal Physiology of the Faculty of Sciences, University of Yaounde 1 (Cameroon). They were housed at a room temperature of about 25 °C in a 12h light/12h dark cycle in the laboratory of animal Physiology of the Department of Biological Sciences of the High Teacher Training College, University of Yaounde 1 (Cameroon). Food and water were available ad libitum. Mice were divided randomly in 6 groups: a normal control group received saline treatment (NaCl 0.9%; per os) (Control), a negative control group received saline treatment (0.9% NaCl; per os) with chronic immobilization stress procedure (NaCl + CIS), one positive control group received Diazepam (2 mg / kg; i.p.) with chronic immobilization stress procedure (Diaz + CIS) and three test groups that received orally different doses of the aqueous extract of *Phragmanthera capitata* with chronic immobilization stress procedure (*Pc* 25 + CIS; *Pc* 125 + CIS and *Pc* 250 + CIS). NaCl 0.9%, Diazepam and the aqueous extract of *Phragmanthera capitata* were administered in a volume of 10 ml/kg of mice body weight. All protocols were performed according to the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the National Ethical Committee of Cameroon (No. FW-IRB00001954).

2.4. Chronic immobilization stress

The aqueous extract was administered per os 30 minutes before the chronic immobilization stress procedure. Mice were subjected to chronic immobilization stress for 10 consecutive days. Chronic immobilization stress consisted of immobilization during 2 h 30 / day in immobilization tubes without access to either food or water [21], [22], [4]. The confinement time (2 h 30 minutes) was divided into two steps separated by an interval of one hour of freedom and the duration of each step was modulated in a variable way in order to avoid adaptation.

2.5. Behavioural Assessments

2.5.1. Open field (OF) test

The OF used was a wooden square box 40 x 40 x 45 cm; the floor was divided into 16 smaller squares of equal dimensions (10 x 10 cm). 24 h after the last chronic immobilization stress procedure, animals received different treatments described above. Thirty minutes after receiving the treatments, animals were placed for 5 min into the centre of the open field [23], [24]. After each test, the box was cleaned with a 19° ethanol solution and wiped dry. Animal behaviours were recorded using a camera (SONY DRC-SR68). The number of crossing (number of square floor units crossed), the time spent in the central square, the number of rearing (number of times the animal stood on its hind legs), the number of grooming, and the number of defecations were evaluated

2.5.2. Light/Dark (L/D) test

The test was performed according to the method described by Campos and colleagues in 2013 [22]. The wood mouse light/dark box consisted of two parts, a light open compartment and a darkened closed compartment. Each mice was placed individually in the centre of the white area facing the opposite site of the entry of the dark compartment and allowed to explore both compartments freely for 5 min. After each test, the box was cleaned with a 19° ethanol solution and wiped dry. Animal behaviours were recorded using a camera (SONY DRC-SR68). Behaviours measured included times spent in light and dark compartment, and latency to first leave (escape) from the light compartment.

2.6. Effects of *Phragmanthera capitata* on the GABAergic neurotransmission and the oxidative stress parameters

Upon exiting the dark/light arena, the animals were sacrificed under anesthesia for biochemical evaluations. The brain was isolated on an ice tray, weighed and crushed in a porcelain mortar, then a TRIS buffer solution (pH: 7.4) was added to obtain a 5% tissue homogenate. The homogenate obtained was centrifuged at 3000 rpm for 25 minutes. The collected supernatant was stored in ependorf tubes at -2 °C in the freezer and the activity level of catalase (CAT) and GABA-transaminase (GABA-T) and concentration of reduced glutathione (GSH), malondiadéhyde (MDA), gamma amino butyric acid (GABA) were evaluated according to different protocols.

2.6.1. GABA Level

The quantity of gamma-amino butyric acid in the homogenate was evaluated by the colorimetric assay technique described by Lowe and colleagues in 1958 [25]. The working reagent consisted of a mixture of 0.2 ml of ninhydrin solution (0.14M) prepared in a carbonate-bicarbonate buffer solution (0.5M; pH 0.9) to which 0.1 ml of glacial trichloroacetic acid (TCA) 10%. 100 μL of the supernatant is then taken and introduced into the previous reagent, then this mixture is incubated at 60 °C for 30 minutes in a water bath. The mixture is allowed to cool and added to a tube containing 2 ml of 0.16% disodium tartrate solution, 0.03% copper sulphate and 0.031% tartaric acid (1/50 dilution). The whole was kept at a temperature of 25 °C for 10 minutes. A standard GABA solution was prepared in parallel from different masses of GABA (100μg, 150μg, 200μg, 300μg, and 400μg) each mixed with 0.15g of glutamate dissolved in 0.5 ml of glacial trichloroacetic acid (10%). The absorbance, measured (377/530 nm) using a spectrophotometer, is proportional to the concentration of GABA in the homogenates

2.6.2. GABA-T activity

The concentration of GABA transaminase was evaluated by the colorimetric assay method of Nayak and Chatteye in 2001 [26]. 15μmol of α-oxoglutarate, 15μmol of GABA and 10μmol of pyrodoxal phosphate are introduced into dry tubes. 0.1 ml of homogenate is added to the test tube and 0.1 ml of 5% methanol to the blank tube. The total volume of the mixture was made up to 3 ml with a TRIS-HCL buffer solution (50mM; pH 7.4). The tubes are incubated at 37 °C in a water bath for 60 minutes. The reaction is finalized by adding 5 ml of glacial TCA 20%. Before reading on the spectrophotometer, 0.1 ml of ferric chloride III (12%) is added. The semialdehyde succinic acid produced during the incubation of the mixture was estimated by spectrometry and the absorbance read at 610 nM after 30 and 90 seconds.

2.6.3. GSH level

The method used was developed by Ellman in 1959 [27] for determining the amount of reduced glutathione based on the fact that dinitro-2,2'-dithio-5,5'-dibenzoic acid (DTNB) reacts with the thiol groups of glutathione to form a complex with a yellow colour which absorbs at 412nM. The pattern (50 µL) was incubated with 750 µL of ellman's reagent. The yellow colour developed after one hour was read in the spectrophotometer at 412 nM.

2.6.4. MDA level

The procedure is that described by Wilbur and colleagues in 1949 [28]. In the test tubes were introduced 300µL of the pattern from brain tissue and in the control tube, 150µL of Tris-HCl buffer 50mM, KCl 150mM, pH 7.4. To each tube was added 150µL of TCA 20% and 300µL of thiobarbituric acid (TBA) 0.67%. The tubes were capped with glass beads and incubated for 10 minutes at 90 °C in a water bath. They were then cooled in tap water and centrifuged at 3000 rpm for 15 minutes at room temperature. The supernatants were decanted and the optical densities read at 530nM.

2.6.5. Catalase activity

Catalase activity was measured using the method of Sinha [29]. For the tissue catalase assay, 12.5µL of pattern and 187.5µL of phosphate buffer (0.1M; pH 7.5) were added to each test tube. The stopwatch was started after adding 50µL of hydrogen peroxide (50mM). After one minute, the reaction was stopped by adding 500µL of dichromate/acetic acid solution. In the control tube was placed 12.5µL of sample and 237.5µL of phosphate buffer (0.1M; pH 7.5) and 500µL of potassium dichromate, the whole was heated to 100 °C for 10 minutes. After cooling, the optical density was read on a spectrophotometer (GENESYS 20) at 570nm. For each tube, the amount of hydrogen peroxide remaining in the solution after adding the acid was evaluated.

2.7. Acute toxicity (LD_{50}) test

The acute systemic toxicity of *Phragmanthera capitata* evaluated the adverse effects that occur following oral exposure of mice to a single dose of the aqueous extract of *Phragmanthera capitata* within 24 hours [30]. This test was carried out in two phases:

2.7.1. Phase I

In the first phase, mice divided into three groups of six mice each, are given 10, 100, 1000 mg / kg of the aqueous extract of *Phragmanthera capitata*. After administration, observations were made at regular interval to check for the onset of adverse effect, time to death or time to recover. The period of observation in this phase I was to 24 hours.

2.7.2. Phase II

This phase involved the use of two groups of six mice each. In this phase, the animals were administered higher dose of 1600, 2900 and 4000 mg/kg of the aqueous extract of *Phragmanthera capitata*. The mice were also monitored for 24 hours. The number of deaths was recorded and the LD_{50} was calculated as the geometric mean of the highest non-lethal dose (a) and the least toxic dose (b).

$$LD50 = \sqrt{a \times b}$$ [30], [31], [32].

2.8. Statistical analysis

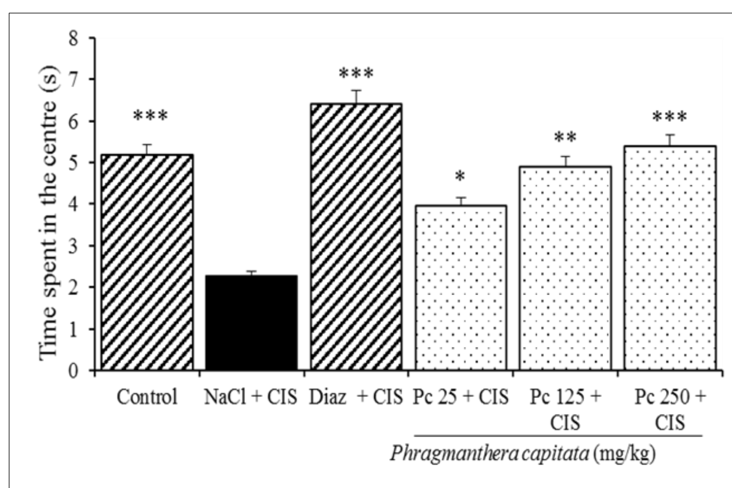
Statistical analysis of the values obtained and the construction of the graphs was performed using Graph Pad Prism version 5.03 and Microsoft Office Excel 2013. The results were expressed as mean ± standard error of mean (SD). The different values were compared using one-way analysis of variance (ANOVA) and when differences existed, the Dunnets's multiple comparison tests were used as the post hoc test. $P \leq 0.05$, data were considered significantly different.

3. Results

3.1. Effects of *Phragmanthera capitata* on anxiety-like behaviours induced by chronic immobilization stress in mice in the open field test

3.1.1. Effects of Phragmanthera capitata on time spent in the centre

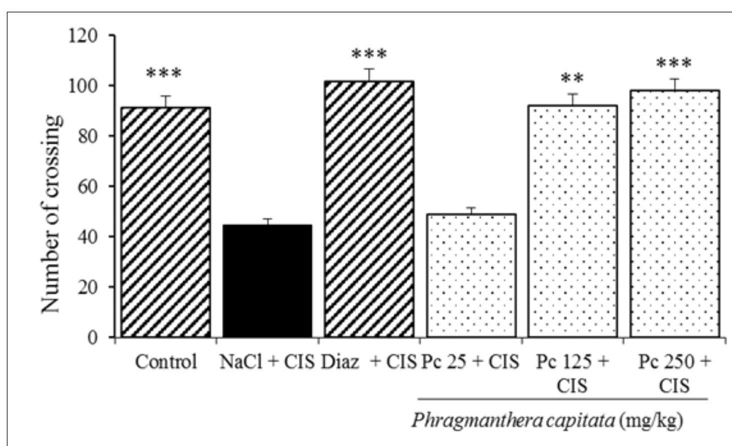
The OF test revealed that CIS induce a significant decrease of the time spent in the centre from 5.18 ± 1.67 in control group to 2.27 ± 0.97 in NaCl + CIS group (56.17%) (Fig 1). *P. capitata* reduced, in dose dependent manner, the decrease induced by the CIS on the time spent in the centre from 5.18 ± 1.67 in control group to 3.94 ± 2.06 in *P.c.* 25 + CIS group (23.93%) and to 4.90 ± 1.76 in *P.c.* 125 + CIS group (5.40%). These effects highlight the increase of the time spent in the centre from 2.27 ± 0.97 in NaCl + CIS group to 3.94 ± 2.06 in *P.c.* 25 + CIS group and to 4. 90 ± 1.76 in *P.c.* 125 + CIS group (Fig 1).



Data are mean of time spent in the centre ± SD on each group. N = 6; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control. Pc 25, Pc 125, Pc 250: doses of *Phragmanthera capitata.*

Figure 1 Effects of *Phragmanthera capitata* on the time spent in the centre

3.1.2. Effects of Phragmanthera capitata on the number of crossing



Data are mean of the number of crossing ± SD on each group. N = 6; ** $p < 0.01$; *** $p < 0.001$ significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control. Pc 25, Pc 125, Pc 250: doses of *Phragmanthera capitata*

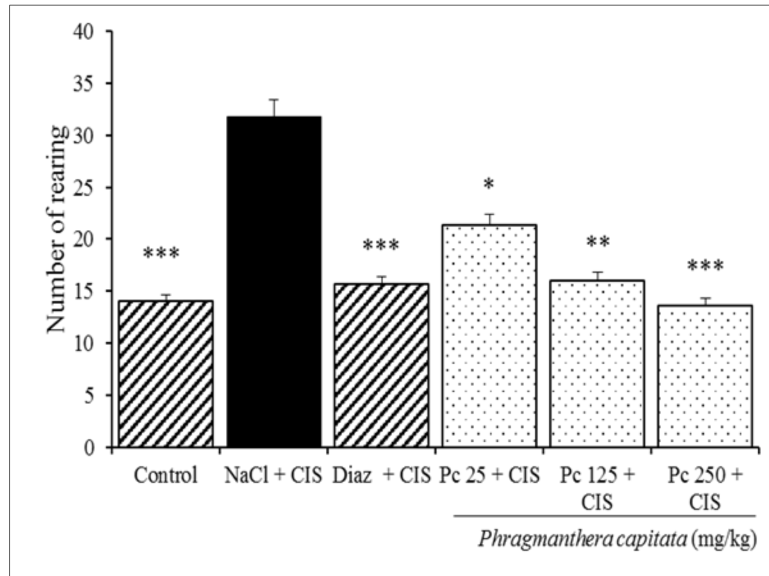
Figure 2 Effects of *Phragmanthera capitata* on the number of crossing

CIS induced a significant decrease of the number of crossing from 91.16 ± 6.88 in control group to 44.66 ± 11.36 in NaCl + CIS group (51%) (Fig 2). The Fig 2 shows that *P. capitata* inhibited the decrease of the number of crossing induced by the CIS from 91.16 ± 6.88 in control group to 49.00 ± 9.16 in *P.c.* 25 + CIS group (46.24%). These effects are underlined

by the increase of the number of crossing from 44.66 ± 11. 36 in NaCl + CIS group to 49.00 ± 9.16 in *P.c.* 25 + CIS group (9.71%) and to 92.16 ± 11.16 in *P.c.* 125 + CIS group (106.35%).

3.1.3. Effects of Phragmanthera capitata on the number of rearing

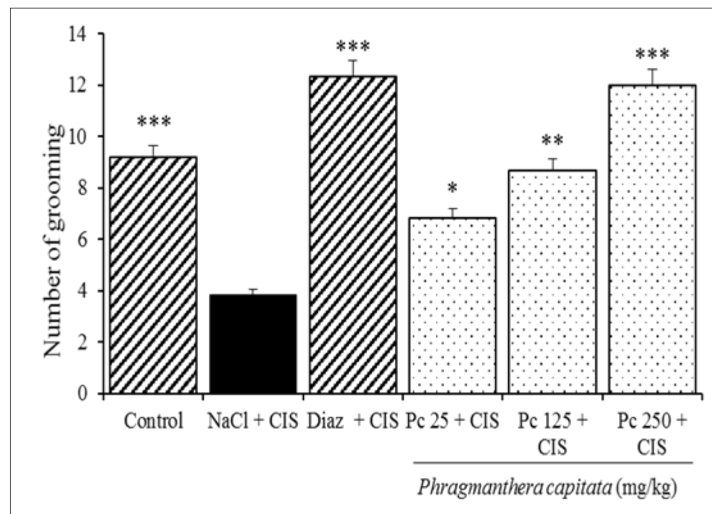
The OF test demonstrated that CIS induced a significant increase of the number of rearing from 14.00 ± 3.68 in control group to 31.83 ± 7.83 in NaCl + CIS group (127.35%). *P. capitata* minimized the increase of the number of rearing induced by the CIS from 14.00 ± 3.68 in control group to 21.33 ± 5.60 in *P.c.* 25 + CIS (52.35%) and to 16.00 ± 8.76 in *P.c.* 125 + CIS group (14.28%); these effects are brought out by the decrease of the number of rearing from 31.83 ± 7.83 in NaCl + CIS group to 21.33 ± 5.60 in *P.c.* 25 + CIS (32.98%) and to 16.00 ± 8.76 in *P.c.* 125 + CIS group (49.73%) (Fig 3).



Data are mean of the number of rearing ± SD on each group. N = 6; * p < 0.05, ** p < 0.01, *** p < 0.001 significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control. *Pc 25, Pc 125, Pc 250*: doses of *Phragmanthera capitata*.

Figure 3 Effects of *Phragmanthera capitata* on the number of rearing

3.1.4. Effects of Phragmanthera capitata on the number of grooming



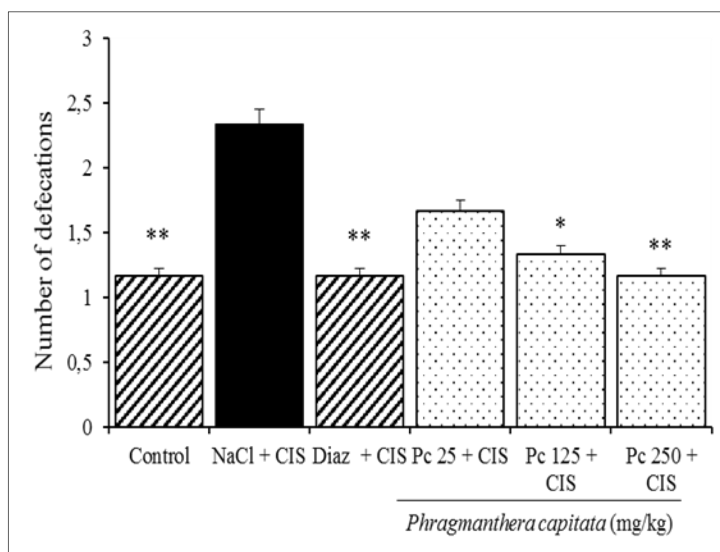
Data are mean of the number of grooming ± SD on each group. N = 6; * p < 0.05; ** p < 0.01; *** p < 0.001 significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control. *Pc 25, Pc 125, Pc 250*: different doses of *Phragmanthera capitat*

Figure 4 Effects of *Phragmanthera capitation* the number of grooming

CIS induced a significant decrease of the number of grooming from 9.16 ± 0.75 in control group to 3.83 ± 1.16 in NaCl + CIS group (58.18 %) (Fig 4).The Fig 4 shows that *Phragmanthera capitata* reduced, in dose dependent manner, the decrease induced by the CIS on the number of grooming 9.16 ± 0.75 in control group to 6.83 ± 1.47 in *P.c.* 25 + CIS group (25.43 %) and to 8.66 ± 1.63 in *P.c.* 125 + CIS group (5.45 %); these effects are displayed by the increase of the number of grooming from 3.83 ± 1.16 in NaCl + CIS group to 6.83 ± 1.47 in *P.c.* 25 + CIS group (78.32 %) and to 8.66 ± 1.63 in *P.c.* 125 + CIS group (126.10 %) (Fig 4).

3.1.5. Effects of Phragmanthera capitata on the number of defecations

The Fig 5 shows that CIS induced a significant increase of the number of defecations from 1.16 ± 0.40 in control group to 2.33 ± 0.51 in NaCl + CIS group (100.86%). *P. capitata* reduced, the increase of the number of defecation from 1.16 ± 0.40 in control group to 1.66 ± 0.81 in *P.c.* 25 + CIS group (43.10%) and to 1.33 ± 0.51 in *P.c.* 125 + CIS group (14.65%); these effects are proven by the decrease of the number of defecation from 2.33 ± 0.51 in NaCl + CIS group to 1.66 ± 0.81 in *P.c.* 25 + CIS (28.75%) and to 1.33 ± 0.51 in *P.c.* 125 + CIS group (42.91%) (Fig 5).



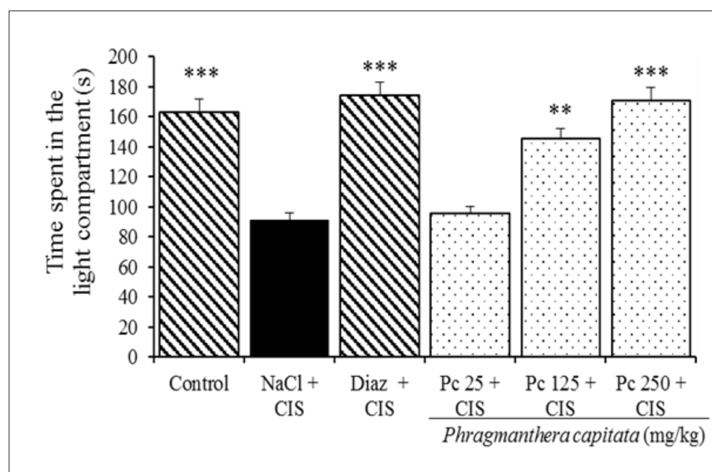
Data are mean of the number of defecation ± SD on each group. N = 6; * $p < 0.05$; ** $p < 0.01$ significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control.

Figure 5 Effects of *Phragmanthera capitata* on the number of defecations

3.2. Effects of *Phragmanthera capitata* on Anxiety-Like behaviours induced by chronic immobilization stress in mice on the Light/Dark Test.

3.2.1. Effects of Phragmanthera capitata on the time spent in light compartment

The L/D test revealed that the CIS induced a significant decrease of the time spent in the light compartment from 163.32 ± 8.81 s in control group to 91.37 ± 8.11 in NaCl + CIS group (44.05%) (Fig 6). *P. capitata* reduced in dose dependent manner the decrease of the time spent in light compartment induced by CIS from 163.32 ± 8.81 s in control group to 95.70 ± 8.88 in *P.c.* 25 + CIS group (41.40%) and to 145.14 ± 29.79 in *P.c.* 125 + CIS group (11.13%). These effects are displayed by the increase of the Time spent in light compartment from 91.37 ± 8.11 in NaCl + CIS group to 95.70 ± 8.88 in *P.c.* 25 + CIS group (4.73%) and to 145.14 ± 29.79 in *P.c.* 125 + CIS group (58.84%) (Fig 6)

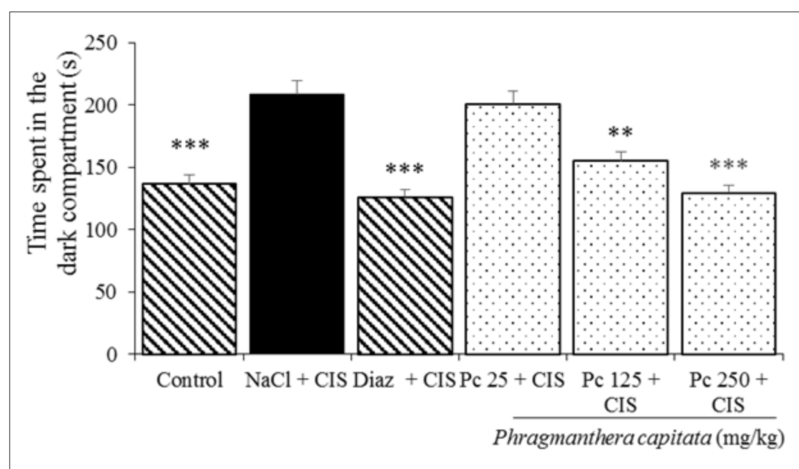


Data are mean of time spent in light compartment ± SD on each group. N = 6; ** $p < 0.01$, *** $p < 0.001$ significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control.

Figure 6 Effects of *Phragmanthera capitata* on time spent in the light compartment

3.2.2. Effects of Phragmanthera capitata on time spent in the dark compartment

The L/D test showed that the CIS induced a significant increase of time spent in dark compartment from 136.67 ± 9.85 s in control group to 208.61 ± 9.07 in NaCl + CIS group (52.63%) (Fig 7). *P. capitata* reduced in dose dependent manner the increase of the time spent in dark compartment induced by CIS from 136.67 ± 9.85 s in control group to 154.85 ± 33,3 s in *P.c.* 125 + CIS group (13.30%). These effects are displayed by the decrease of the time spent in dark compartment from 208.61 ± 9.07 in NaCl + CIS group to 154.85 ± 33,3 s in *P.c.* 125 + CIS group (25.77%) and to 129.19 ± 16.26 in *P.c.* 250 + CIS group (38.07%) (Fig 7).

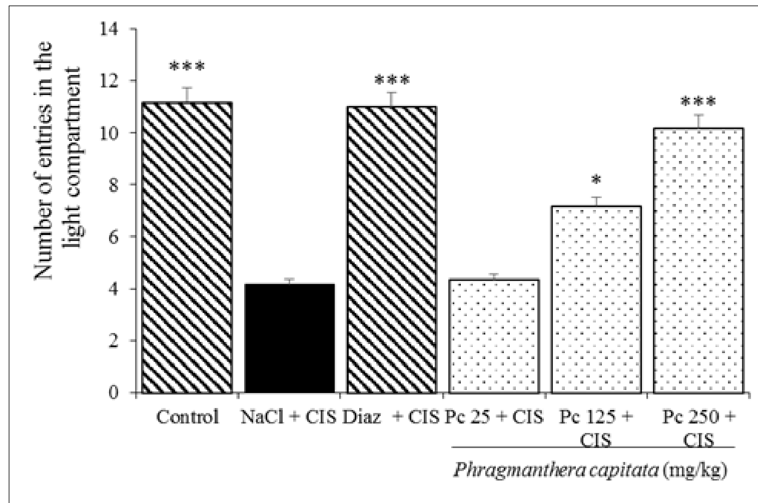


Data are mean of time spent in light compartment ± SD on each group. N = 6; ** $p < 0.01$, *** $p < 0.001$ significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control.

Figure 7 Effects of *Phragmanthera capitata* on time spent in dark compartment

3.2.3. Effects of Phragmanthera capitata on the number of entries in the light compartment

CIS induced a significant decrease of the number of entries in light compartment from 11.16 ± 1.94 in control group to 4.16 ± 1.72 in NaCl + CIS group (62.72%) (Fig 8). *P. capitata* reduced, in dose dependent manner, the effects of CIS on the number of entries in light compartment. These effects are underlined by the increase of the number of entries in light compartment from 4.16 ± 1.72 in NaCl + CIS group to 7.16 ± 1.47 in *P.c.* 125 + CIS (72.11%) and to 10.16 ± 1.72 in *P.c.* 250 + CIS group (144.23%) (Fig 8).

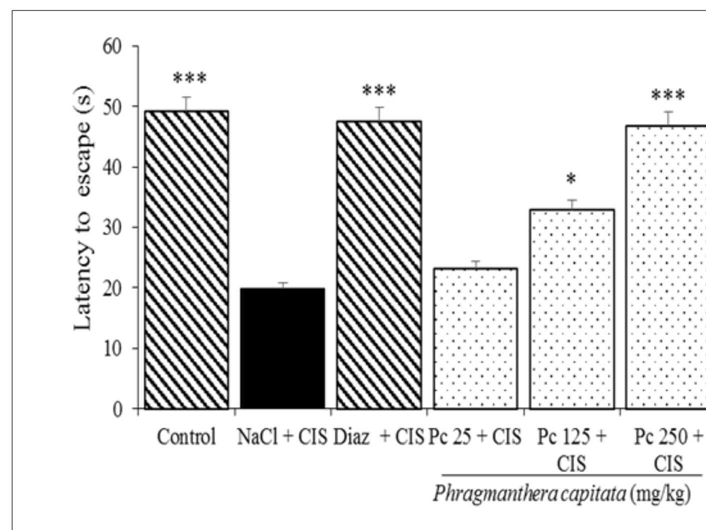


Data are mean of time spent in light compartment ± SD on each group. N = 6; ** p < 0.01, *** p < 0.001 significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control.

Figure 8 Effects of *Phragmanthera capitata* on the number of entries in the light compartment

3.2.4. Effects of Phragmanthera capitata on the latency to escape the light compartment

CIS induced a significant decrease of the latency to first escape from 49.16 ± 7.57 s in control group to 19.83 ± 4.57 s. in NaCl + CIS group (59.66%) (Fig 9). *P. capitata* reduced, in dose dependent manner, the effects of CIS on the latency to first escape the light compartment. This lowering effect is proven by the increase of the latency to first escape from 19.83 ± 4.57 s in NaCl + CIS group to 23.16 ± 7.05 s in *P.c.* 25 + CIS (16.79%) and to 32.83 ± 6.46 s in *P.c.* 125 + CIS group (65.55%) (Fig 9).



Data are mean of latency to first escape the light compartment ± SD on each group. N = 6; ** p < 0.01; *** p < 0.001 significant differences in comparison with NaCl + CIS group. NaCl + CIS = negative control; Diaz + CIS = positive control.

Figure 9 Effects of *Phragmanthera capitata* on the latency to first escape the light compartment

3.3. Effects of *Phragmanthera capitata* on the GABAergic neurotransmission

3.3.1. Effects of Phragmanthera capitata on the level of GABA

The GABA level decreased from 397.60 ± 8.72 µg/g tissue in control group to 139.72 ± 8.73 µg/g tissue (p < 0.001) in the NaCl + CIS group. *P. capitata* induced a dose-dependent increase in GABA level. The highest dose of *P. capitata* (250mg/kg) induced an increase up to 393.96 ± 4.84 µg/g tissue (p < 0.001) compared to the NaCl + CIS group. The GABA level was also increased in diazepam group (p < 0.001), compared to the NaCl + CIS group (Table 1).

3.3.2. Effects of Phragmanthera capitata on the activity of GABA-T

GABA-transaminase activity increased from 47.85 ± 7.34 pg/ min/mg tissue in control group to 100.65 ± 7.34 pg/min/mg tissue ($p < 0.001$) in NaCl + CIS group. *Phragmanthera capitata* has diminished the GABA-T activity from 100.65 ± 7.34 pg/min/mg tissue in the NaCl + CIS group to 75.90 ± 8.55 ($p < 0.005$) and 43.89 ±7.12 pg/min/mg tissue ($p < 0.001$) in *Phragmanthera capitata* 125 + CIS group and *Phragmanthera capitata* 250 + CIS group respectively. Diazepam also reduced GABA-T activity to 50.16 ± 8.91 pg/min/mg tissue ($p < 0.001$) compared to the NaCl + CIS group (Table 1).

3.4. Effects of *Phragmanthera capitata* on oxidative stress parameters

3.4.1. Effects of Phragmanthera capitata on the activity of CAT

Catalase activity significantly increased from 151.93 ± 4.46 kU/g of tissue in the control group to 283.66 ± 5.83 kU/g tissue in the NaCl + CIS group ($p < 0.001$). *P. capitata* reversed the increase in Catalase activity from 283.66 ± 5.83 kU/g in NaCl+ CIS group to 250.22 ± 26.25 kU/g ($p < 0.05$) and 237.43 ± 30.54 kU/g of tissue ($p < 0.005$) in *P. capitata* 125 + CIS group and *P. capitata* 250 + CIS group respectively. Diazepam also reversed the increase of Catalase activity induced by CIS ($p < 0.001$; Table 1).

3.4.2. Effects of Phragmanthera capitata on the level of GSH

The GSH level decreased significantly in the NaCl + CIS group (106.48 ± 6.36 nmol/mg tissue) compared to the control group (210.73 ± 3.50 nmol/mg tissue; $p < 0.001$). *P. capitata* induced a dose-dependent a reduction of the decrease of the GSH level from 106.48 ± 6.36 nmol/mg of tissue in the NaCl + CIS group to 165.81 ± 10.70 ($p < 0.001$) and 210.08 ± 7.44 nmol/mg of tissue ($p < 0.001$) in *P. capitata* 125 + CIS group and *P. capitata* 250 + CIS group respectively. Diazepam increased GSH level to 209.93 ± 9.52 nmol/mg ($p < 0.001$), respectively, relative to the NaCl + CIS group (Table 1).

3.4.3. Effects of Phragmanthera capitata on the level of GSH

MDA level increased significantly from 127.54 ± 1.14 nmol/g tissue in the control group to 177.83 ± 9.28 nmol/g tissue in the NaCl + CIS group ($p < 0.001$). *P. capitata* reversed the increase in MDA level from 177.83 ± 9.28 nmol/g in NaCl + CIS group to 160.33 ± 9.89 nmol/g ($p < 0.005$) and 138.03 ± 6.43 nmol/g of tissue ($p < 0.001$) in *P. capitata* 125 + CIS group and *P. capitata* 250 + CIS group respectively. Diazepam also reversed the increase of MDA induced by CIS ($p < 0.001$; Table 1).

Table 1 Effects of *P. capitata* on stress markers in brain of mice after CIS

Traitments Groups	**Doses (mg/kg)**	**GABA (µg/g)**	**GABA-T (pg/min/mg)**	**GSH (mmol/mg)**	**MDA (mmol/mg)**	**CAT (kU/g)**
Control	-	397.6 ± 8.72	47.85 ± 7.34	210.73 ± 3.50	127.54 ± 1.14	151.93 ± 4.46
NaCl + CIS	-	139.72 ± 8.73	100.65 ± 7.34	106.48 ± 6.36	177,83 ± 9.28	283.66 ± 5.83
Diaz + CIS	2	390.50 ± 7.86	50.16 ± 8.91	209.93 ± 9.52	124,95 ± 3.90	153.11 ± 9.12
P. capitata 25 + CIS	25	210.09 ± 9.67	95.37 ± 5.22	122.71 ± 11.01	174,75 ± 8.71	250.22 ± 26.25
P. capitata 125 + CIS	125	285.88 ± 5.52	75.90 ± 8.55	165.81 ± 10.70	160.33 ± 9.89	237.43 ± 30.54
P. capitata 250 + CIS	250	393.96 ± 4.84	43.89 ±7.12	210.08 ± 7.44	138.03 ± 6.43	174.23 ± 23.72

Data are means ± SD. N = 6 per dose. Control : distilled water

3.5. Acute toxicity of *Phragmanthera capitata*

No death was recorded up to dose 2900 mg/kg following the administration dose range of our protocol. The first cases of death were recorded at the dose 4000 mg/kg (3deaths over 6 mice). Thus, 2900 mg/kg was considered as the highest

non-lethal dose (a) and 4000 mg/kg was considered the least toxic dose (b). The estimated LD_{50} was then 3405.87 mg/kg.

4. Discussion

Upshots of this study clarify the effects of *Phragmanthera capitata* on anxiety-like behaviours induced by CIS in mice. The time spent in the centre of the open field apparatus indicates immobility behaviour. Our study showed that CIS induces a significant decrease of the time spent in the centre, demonstrating that anxiety reduces immobility in mice. The administration of *Phragmanthera capitata* induced an increase of immobility similarly to the orthodox treatment with diazepam, suggesting anxiolytic effect. The number of crossing refers to exploratory behaviour in this test. The administration of *Phragmanthera capitata* induced an increase of exploratory activity on anxious mice. The increase in the number of crossing is a sign of intrinsic inhibition of anxiogenesis [33]. The number of rearing indicates locomotion behavior in this test. The administration of *Phragmanthera capitata* on anxious mice induced a decrease of exploration. Because of the direct relation between exploration and anxiety, this result highlights an exploration decreasing effects, so a sign of intrinsic inhibition of anxiogenesis, of *Phragmanthera capitata* on anxious mice [33]. Grooming behaviour is an index of behavioural adaptation to a stressful situation. Findings of this study in the open field test show an increased number of grooming on animals treated with *Phragmanthera capitata*, indicating an anxiolytic-like effect according to the similar reduction of anxiety-like behaviour observed following the orthodox treatment with diazepam [34]. The number of defecations refers to excretion. In this study, as Fig. 5 shows that anxiety increases the number of defecation, it indicates also that *Phragmanthera capitata* induced a reduction of the number of defecation on anxious mice. Defecation is an indicator of emotionality in animals; research shows that high emotionality is related to an increase in defecation and that anti-anxiety drugs reduce defecation in anxious mice [34]. This result suggest an excretory lowering, so an anxiolytic, effect of *Phragmanthera capitata.*

An increase of the number of crossing and time spent in the centre of the open field displays a reduction of stress [35],[35], [37]. Therefore, it is known that anxiolytic treatments do not by themselves increase exploration in the open field but they decrease the stress-induced reduction of exploration behaviour [38]. open field paradigm findings suggests that this plant possesses anxiolytic and or anti anxiogenic properties.

The light/dark (L/D) test is a commonly used model to evaluate anxiolytic-like effects in rodents [39]. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of the animals [40]. In rodents, this model generates an inherent conflict between their exploration and their avoidance of the light compartment. *P. capitata* increased the time spent in the light compartment of the L/D paradigm in stressed mice. Treatment with anxiolytic drugs such as benzodiazepines increases the time spent in the light compartment as well as the number of transitions between the two areas [22] so the increase of the time spent in the light compartment suggests an anxiolytic effect. It is known that treatment with anxiolytic, such as Diazepam, increases the time spent in the light compartment [40]. The light/dark outcomes confirm anxiolytic-like effects of *P. capitata* observed in the open field. These findings are in accordance with those of Takem and colleagues [20] that showed an anxiety lowering potential of *P. capitata.* Several psychiatric and neurological disorders including anxiety are associated with low GABA levels or decreased GABA function in the brain. Too much nervous excitation can lead to neuropsychiatric diseases; it must be balanced with inhibition. GABA, the most important inhibitory neurotransmitter in the brain, provides this inhibition by acting during stress. Many anti-anxiety treatments target the GABAergic neurotransmission.. GABA is produced via the action of the enzyme glutamate decarboxylase on glutamic acid and metabolized by GABA-T [48]. The involvement of GABA neurotransmission is supported through the inhibition of the activity of GABA-T by *Phragmanthera capitata* aqueous extracts that also explained the increase of brain GABA concentration in administered mice with the different doses of extracts. These results suggest that *Phragmanthera capitata* aqueous extract is gifted to refurbish and sustain the steadiness between neuronal excitation and inhibition through the modulation of GABAergic neurotransmission, and reduce anxiety in mice [33].

Oxidative stress has been implicated in neurologic degenerative disorders [41]. So exploration of natural elements which have biological activity become one of the researcher targets because synthetic compounds which have biological activities such as synthetic antioxidant compounds are carcinogenic [42]. Degenerative diseases, where the function and structure of a tissue or organs deteriorate over time have been attributed to oxidative stress conditions, supporting that oxidative stress and degenerative diseases are interconnected [43]. Moreover, free radicals are involved in the neurotoxicity and hyper excitability of the amygdale observed during chronic stress. Catalase is present in almost all aerobic organisms; it was the first identified oxidoreductase. Catalase breaks down hydrogen peroxide molecules into water and oxygen. Catalase deficiency or malfunction leads to many diseases such neuropsychiatric diseases. This study showed that *P. capitata* increases catalase level in stressed mice. An increase of Catalase level suggests an increase of antioxidant activity. Similarly, a low level of GSH is the manifestation of oxidative stress because GSH prevents any

cellular deleterious effect such as lipid peroxidation [44] by neutralizing some free radicals. This study also showed that *P. capitata* increases GSH level in stressed mice, suggesting an increase of antioxidant activity. The increase of antioxidant activities is confirmed by the level of MDA. The level of MDA is a marker of lipid oxidation. It is known that MDA is one of the end products of the oxidation of polyunsaturated fatty acids. Therefore, a high level of MDA is an indicator of oxidative stress and cellular damages [45]. This study reveals that *P. capitata* decreases the level of MDA in the brain of depressed mice by increasing antioxidant activities. The decrease of MDA and the increase in GSH and catalase reveal an antioxidant effect of the plant [46]. These findings are in accordance with those of Etame-Loe and colleagues that demonstrate the antioxidant potential of *P. capitata* **Error! Reference source not found.**. The result of acute toxicity evaluation is in accordance with results of Takem and colleagues that showed the safety of the aqueous extract of *Phragmanthera capitata* up to a dose of 3000 mg/kg [18].

5. Conclusion

This study brings out that *P. capitata* increases the GABA level and decreases the GABA-T activity in the brain of stressed mice. The increase in GABA level and a decrease in GABA-T activity highlight an interaction of *P. capitata* with the GABA neurotransmission. These results prove that *P capitata* enhances the GABAergic neurotransmission, illustrating that this plant is able to inhibit anxiogenic effects of CIS mice.

P. capitata possesses anti-anxiogenic and or anxiolytic-like effects mediated by an action on the GABAergic neurotransmission and the antioxidant system. These properties support the pertinence of the use of this plant in Cameroonian traditional medicine

Compliance with ethical standards

Acknowledgments

The authors are very thankful to Lab Animal Physiology of the high teacher training school of the University of Yaounde I, Cameroon, for supporting us by providing apparatus.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest in this study.

Statement of ethical approval

All protocols were performed according to the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the National Ethical Committee of Cameroon (No. FW-IRB00001954).

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