Bridelia atroviridis (Phyllanthaceae) Aqueous Extract Attenuates Scopolamine-induced Amnesia and Depression in Rat: Role of Cholinergic, Monoaminergic, and Antioxidant Mechanisms

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Anti-inflammatory and antioxidant activities are the main pathways of neuroprotective drugs. *Bridelia atroviridis* possesses those activities and is empirically used by Cameroonian traditional healers to alleviate memory and mood disorders.

Objective: The purpose of this study is to evaluate the anti-amnesic and antidepressant effects of *Bridelia atroviridis* aqueous extract (EA).

Methods: Scopolamine was used to induce amnesia and depression in rats. Administrated per os, three doses of EA, including 36, 72, and 144 mg/kg, were used. Behavioral disorders were evaluated through novel object recognition, the Morris water maze, forced swimming, and an open arena. The oxidative stress markers, like malondialdehyde, reduced glutathione, and nitrite levels in the brain, were measured. Neurometabolites, serotonin, and acetylcholine levels were determined. Hematoxylin-eosin paraffin-embedded histological sections were used to assess neuron viability in the cortex and the hippocampus.

Results: Pre-treatments with EA attenuated the deleterious effects of scopolamine and improved brain biomarkers. Indeed, when compared to vehicle control, *Bridelia atroviridis* increased Ca$^{2+}$, Mg$^{2+}$, serotonin, and acetylcholine levels in the brain. Furthermore, the plant reduced significantly (p < 0.001) the recognition index in the novel object recognition test and increased (p < 0.001) the time spent in the target quadrant in the Morris water maze test. Besides, EA has scavenging activities and attenuates neuron death in the cortex and hippocampus.

Conclusion: According to this study, the aqueous extract of *B. atroviridis* barks protects against scopolamine-induced memory loss and depressive disorders. The preventive activities of the plant involve cholinergic and monoaminergic pathways and antioxidant potential.

Graphical Abstract:

Keywords: Memory disorders; depressive behavior; scopolamine; *Bridelia atroviridis*.
1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease of the brain tissue that causes neuronal cell function to deteriorate and causes death [1]. It is the most common cause of dementia, causing memory loss and dysphasia [2,3]. It is not a normal part of aging and can cause a person to become confused, get lost in familiar places, misplace items, or have difficulty communicating. Alzheimer's disease symptoms include a gradual cognitive decline syndrome, short-term memory impairment (recent dialogue, name, or event), indifference, a loss of memory, and other cognitive abilities severe enough to interfere with daily life. The brain's abundance of plaques and tangles causes cognitive impairment. Because Alzheimer's disease affects people differently, each person may experience symptoms differently [4-6]. According to a recent World Health Organization report, dementia affects approximately 50 million people, with nearly 10 million new cases diagnosed each year [3]. The elderly population is growing, as is the prevalence of age-related health issues like cognitive impairment and dementia. Later symptoms include difficulty with time and space, confusion, communication difficulties, personal hygiene issues,eligibility, exercise, and sleeping. Alzheimer's disease is characterized by the accumulation of amyloid protein plaques in neurons. The clinical and evolutionary profiles of the disease can be extremely diverse. Alzheimer's disease has been linked to depression and anxiety in some patients [7-9]. Depression is a mental disorder characterized by episodes of low mood accompanied by a loss of pleasure or interest in normally enjoyable activities [10].

Although there is currently no cure for Alzheimer's disease, medications and symptom management strategies are available [11,12]. The Food and Drug Administration (FDA) in the United States has approved medications that fall into two categories: drugs that slow the progression of Alzheimer's disease and drugs that may temporarily alleviate some symptoms of the disease. Memory impairment treatments include aducanumab, lecanemab, donepezil, and rivastigmine, while depression treatments include imipramine, fluoxetine, and paroxetine [13-16]. They are linked to serious side effects like vomiting, cramps, hallucinations, fatigue, insomnia, loss of appetite, and nausea [17]. As a result, new strategies are required, and herbal medicine is a viable option due to its low risk of side effects [18].

B. atroviridis, a Phyllanthaceae shrub, is used empirically to treat memory disorders, diabetes, malaria, and venereal diseases by Cameroonian traditional healers [19]. The genus Bridelia Willd. (Phyllanthaceae) has 60–70 species that are found in Africa and Asia [20]. According to Terashima and Ichikawa's ethnobotanical study, the juice of soft B. atroviridis leaves is used to treat severe convulsions [21]. Epileptogenesis is well known for “hijacking” normal memory processes [22]. Furthermore, according to Olabisi et al.'s phytochemical analysis, B. atroviridis contains phenolic compounds. It contains flavonoids, beta-carotene, and lycopene, all of which have antioxidant and anti-inflammatory properties [23]. Indeed, recent research has revealed that the phenolic hydroxyl group may contribute to anti-amyloidogenic activity. Phenyl methoxy groups appear to contribute to amyloid peptide suppression [24]. In a nicotinamide-induced model of diabetes in rats, the hypoglycemic, anti-inflammatory, and antioxidant properties of B. atroviridis were highlighted [19].

Given that B. atroviridis was empirically used by indigenous healers in Cameroon to alleviate memory loss and mood disorders, organic molecules with anti-amnesic properties also have antioxidant and anti-inflammatory properties. The present study hypothesizes that Bridelia atroviridis aqueous extract has anti-amnesic and antidepressant properties. This hypothesis was tested using the scopolamine-induced depression and amnesia model in Wistar rats. As a result, the current study sought to assess the anti-amnesic and antidepressant effects of B. atroviridis bark in a scopolamine-induced Alzheimer's disease model in rats. The effects of Bridelia atroviridis aqueous extract were compared to those of donepezil and imipramine.

2. MATERIALS AND METHODS

2.1 Plant Material, Extraction, and Determination of Study Doses

B. atroviridis stem barks were collected in Mbalmayo (Centre, Cameroon) in January 2021. By comparing it to sample N°35241/HNC Cam, a voucher specimen of the plant was authenticated and deposited at Cameroon's National Herbarium (http://sweetgum.nybg.org/science/ih/herbarium-details/?im%25C2%BC125449). The bark was removed, dried in the shade, and ground according to the traditional healer's protocol. For 45 minutes, 38 g of B. atroviridis powder was
boiled in 1 L of tap water. The filtrate was oven dried at 45 °C after filtration through Whatman No. 3 paper to yield 0.825 g of a crude extract with a yield of 2.17%. The doses of aqueous extract given to rats were calculated using the interspecies dose interpolation formula [25]. Humans were given approximately 11.78 mg/kg by the traditional healer. The dose of 72 mg/kg was calculated by multiplying the human dose by the conversion factor for rat metabolism (6.14). Divide and multiply the main dose by 36 mg/kg and 144 mg/kg, respectively. The volume of administration in the current study is based on laboratory animal care guidelines.

2.2 Animal Material and Ethical Statement

The general care of the experimental animals used in this study was done under the Animal Welfare Act. All experiments were carried out following the principles and procedures of the European Union on Animal Care (CEE Council 86/609) as adopted by the Cameroon Institutional National Ethic Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954). All efforts were made to minimize the animals' pain. All animal experiments were carried out under the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines under EU Directive 2010/63/EU for animal experiments. Randomization procedures during the experimentation were performed following the ARRIVE 2.0 guidelines (https://arriveguidelines.org/arrive-guidelines). Healthy albino Wistar rats (10–12 weeks old) weighing 130–150 g were supplied by the Animal Physiology Laboratory at the University of Yaoundé 1 (Cameroon). At room temperature (the natural cycle), all rats were housed in clean plastic cages. They had unrestricted access to running water and soy-free rat chow.

2.3 Reagents and Chemicals

Scopolamine, donepezil, and imipramine are from Sigma Aldrich, Saint Louis, USA. Reagents and chemicals used for biochemical tests were purchased from Sigma Aldrich Co., St. Louis (USA).

2.4 Experimental Design

Forty-nine male rats were randomly assigned to one of seven groups of seven animals, each of which received 30 days of preventive treatment as follows: Groups 1 (normal control) and 2 (negative control) were given distilled water (10 mL/kg) per os, while groups 3 and 4 (positive control) were given donepezil per os (6 mg/kg) and imipramine via intraperitoneal injection (i.p.) (12 mg/kg), respectively. Groups 5, 6, and 7 were given aqueous extracts of *B. atroviridis* at doses of 36 mg/kg, 72 mg/kg, and 144 mg/kg, respectively. 0.9% NaCl and scopolamine (1 mg/kg) were administered daily, 45 minutes after treatment, to the normal control and all other groups, respectively, from day 16 to day 30. From day 19 to day 29, behavioral tests were conducted, and scopolamine administration was stopped. The Morris Water Maze (MWM) and New Object Recognition (NOR) tests were used to assess long-term and short-term memory, respectively. The rats' depressive behavior was assessed 48 hours later using the Forced Swim Test (FS) and Open Arena (OA) tests. The animals were sacrificed at the end of the behavioral tests, and each rat's brain was removed after opening the skull. The brain was divided into two hemispheres. The cortical and hippocampal regions were homogenized using the right hemisphere. The supernatant obtained after centrifugation of the homogenates was used to determine certain biochemical parameters (acetylcholine, acetylcholinesterase, serotonin, MDA, GSH, nitrites, calcium, and magnesium). The left hemisphere was used to perform histological analysis of the cortex and hippocampus (Fig. 1).

2.5 Behavioral Tests

**New Object recognition (NOR) test:** Ennaceur and Delacour developed the object recognition test in 1988 to assess episodic memory. This test is based on rodents' natural preference to explore unfamiliar objects over familiar ones [26]. Simply placing the animal in a new environment causes attentional arousal, which leads to intense exploration. The object recognition test is a particularly appropriate test for assessing hippocampal-dependent memory processes in rodents because it focuses on rodents' natural proclivity to explore new stimuli while avoiding other variables that could influence the results. The test was conducted in three stages, the first of which was the habituation phase, in which the animals were placed individually in turn in the arena for 5 minutes on the first day. The second was the training phase. On the second day, the rats were individually presented with two identical objects for a 5-minute session to familiarize themselves with the objects. The last one is the evaluation phase. Here, the rats were presented with two objects 24 hours after the training phase, one of which had been used in the familiarization phase and the other new to them.
Fig. 1. Experimental chronogram of the study

**Morris Water Maze (MWM) test:** It is based on the animal’s swimming ability and its natural desire to escape from water that is unpleasant to it. A circular brown metal enclosure, 150 cm in diameter and 60 cm deep, filled to a depth of 40 cm with room-temperature water, was used in this work. This enclosure also included a platform measuring 39 cm. The MWM test was carried out in two stages:

- **A four-day training period**

  During this stage, the animals were dropped into the water from the device’s edge. They had to swim to the platform, the only place where they could get out and stand if they wanted to get out of the water. Each rat was placed in the pool sequentially in the other three quadrants (at the pool's periphery and facing the tank wall) for each training trial. They had a maximum of 60 seconds to locate the platform. Rats that found the platform were allowed to stay for 10 seconds. They were then wiped down with a dry cloth and returned to their cage to rest before the next test. Those who were unable to find the platform within 60 seconds were directed by the experimenter. They stayed on the platform for ten seconds. Three training trials were conducted per day for four days in a row, and the water was opacified with milk powder on the second day.

- **Test phase**

  The platform was removed after four days of training, and each rat was allowed to swim for 60 seconds inside the tank. As a result, the animal that knew where the platform was supposed to be was supposed to be looking for it there. The time it took the animal to find the original platform position, the number of entries in the target dial, and the time spent in the target dial were the parameters assessed.

**Forced swimming (FS) test:** This is a rodent depression assessment test described by Estrada-Camarena et al. [28]. A single rat was placed in a water tank 50 cm high and 30 cm wide for the test. These dimensions ensured that the rat could not escape by clinging to the device’s edges. The tank was filled with room-temperature water. The water was 35 cm deep to prevent the rat from using its lower limbs to hold onto the surface, forcing it to swim. When the animal floated horizontally and made only small movements to keep its head above water, it was considered immobile. The FS test is divided into two phases separated by a 24-hour interval: a 15-minute pre-test phase and a 5-minute test phase during which immobility and swimming times are recorded.

**Open arena (OA) test:** The Open Arena Test (OA) measures rodent locomotor activity, exploration, and emotional reactivity [29]. The OA consists of a square enclosure with sharp edges. The exploration surface is divided into 17 tiles: 16 on the inner surface of the experimental setup and 1 in the center. The open arena measured 165 cm long, 165 cm wide, and 83 cm high. The animals were placed in turns in the center of the arena, and after 5 minutes of exploration, the number of lines or tiles crossed was calculated. The number of times the animal stood on its hind legs while resting its front legs on the arena walls, and the number of times the animal cleaned itself.

2.6 **Preparation of Tissue Samples**

The animals were anesthetized with ether and then sacrificed by decapitation at the end of the behavioral tests (day 30) and after a 12-hour non-hydrous fast. Brains were removed from the skull using fine scissors and weighed with a
balance (Mettler PL 301). Each brain’s hemispheres were separated. The left brains were stored in sterilized urine pots containing 10% buffered formalin for subsequent histological sections, while the right hemispheres were used for homogenates. The right brain’s hippocampal region and cortex were collected separately and ground in a glass mortar; 2 mL of tris buffer (0.1 M pH = 7.5) was then added. The mortar and its contents were placed in an ice tray during grinding. The collected supernatant was stored at -20 °C after centrifugation at 3000 rpm for 15 minutes.

2.7 Biochemical Assays

Assessment of calcium and magnesium levels: Brain calcium and magnesium levels were assessed using commercial diagnostic kits from Biolabo and Randox, respectively. Calcium concentration was determined by its reaction with o-cresol phthalein complexon by the protocol of Baginski et al. [30]. For magnesium level, it was determined by its reaction with the xylidyl blue complex by the protocol of Treiz [31].

Assessment of neuromodulators’ activity or concentrations: The serotonin (5-HT) level was determined using the methods described by Yoshitake et al. [32]. Indeed, 5-HT levels were determined using column liquid chromatography with fluorescence detection after derivatization with benzyl amine and 1,2-diphenyl ethylenediamine. Furthermore, the Hestrin [33] protocol was used to react acetylcholine and other carboxylic acid derivatives with hydroxylamine.

Assessment of malondialdehyde (MDA) level: Malondialdehyde (MDA) causes the formation of aldehydes. Wilbur et al.’s method was used to determine the MDA concentration [34]. Aldehydes react with thiobarbituric acid (TBA) to form a pink complex that absorbs at 530 nm. The density of the pink color is proportional to the concentration of MDA in the homogenate. To perform this assay, 250 L of homogenate was added to the test tubes, while 250 L of Tris buffer (60 mM HCl; 150 mM KCl; pH 7.4) was added to the blank tube. Each tube received 125 L of 20% trichloroacetic acid (TCA) and 250 L of 0.67% TBA. The mixture was incubated for 10 minutes at 90 degrees Celsius, cooled to room temperature, and centrifuged at 4000 revolutions per minute for 15 minutes at room temperature. A spectrophotometer set to 530 nm was used to measure supernatant absorbance against a blank. The MDA concentration was expressed in mol/g of tissue.

Assessment of reduced glutathione (GSH) level: Ellman (1959) [35] described a method for determining the concentration of GSH. The Ellman reagent (2,2-dithio-5,5-dinitrobenzoic acid) bonds with glutathione SH groups in the homogenate. This reaction produces a yellow complex with a 412 nm absorbance. 1.5 mL of Ellman reagent was added to tubes already containing 100 L of homogenate or 100 L of Tris buffer (50 mM HCl; 150 mM KCl; pH 7.4) for the blank tube for this assay. The tubes were then vortexed and incubated at room temperature for 1 hour. A spectrophotometer was used to measure absorbance at 412 nm in comparison to a blank. The GSH level was calculated as µmol/g tissue.

Assessment of nitrite level: Nitrite diazotize in the presence of amino-4-benzenesulfonamide (sulfanilamide) and N-(naphthyl-1,1)-diamino-1,2-ethane dichloride (N-1-naphthyl ethylenediamine) in acid medium. The product is proportional to the concentration of nitrite in the sample. Using the method described by Green et al. [36], the contents of each tube were homogenized and incubated in the dark for 10 minutes at room temperature. Absorbance was read against the blank with a spectrophotometer at 546 nm. The nitrite level was calculated as µmol/g tissue.

2.8 Histological Analysis and Neuron Counting

The hippocampal region was fixed in 10% buffered formalin for 2 weeks before being trimmed and dehydrated in croissant gradient alcohol (70%, 80%, 90%, and 100% (3 baths)). Tissues were clarified in two xylene baths (1 hour and 30 minutes per bath) before being impregnated in liquid paraffin at 60 °C for five hours. The number of hippocampal neurons in the CA1 and CA3 regions was determined using microscopy obtained with a light microscope (Leitz Wetzlar, Germany, 513). The optic microscope was connected to a digital camera (Celestron 44421) linked to a computer. The images captured by the DCM35 software were transferred and analyzed using the Image J software.

2.9 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8.0.1. Data were expressed as
an average ± Standard Error on Mean (SEM) or as percentages. They were compared using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A difference in values was considered statistically significant at p < 0.05.

3. RESULTS

3.1 Effects of the Aqueous B. atroviridis Extract (EA) on Short-term Memory

To investigate EA’s effects on short-term memory, the New Object Recognition Test (NOR) was carried out. Fig. 2 shows the effects of treatment with an aqueous extract of B. atroviridis on the recognition index (RI). The RI was significantly reduced (p < 0.01) by 21.53% in rats treated solely with scopolamine compared to the scopolamine-treated control. The preventive treatment with the extract (36 mg/kg) resulted in a significant (p < 0.001) increase in the RI of 44.28% compared to the negative control lot. Preventive treatment with EA (72 mg/kg) resulted in a significant (p < 0.01) increase in the RI of 23.09% compared to the vehicle control. Preventive treatment with donepezil resulted in a significant increase (p < 0.01) in the RI of 23.84% compared to the vehicle control.

3.2 Effects of the Aqueous Extract of B. atroviridis on Depressive Behaviors

To determine EA on long-term memory, the latency time and the time spent in the target quadrant in the Morris Water Maze Test (MWM) were evaluated (Fig. 3). Scopolamine-treated animals slowly located the target quadrant. The latency time was 2.5 times greater than the normal control. In rats pretreated with an aqueous extract of B. atroviridis, we observed a significant decrease in latency time compared to the negative control. This decrease was 55.93% (p<0.05) and 59.32% (p<0.001) for the 36 and 72 mg/kg extract doses, respectively. Preventive treatment with donepezil and aqueous extract (144 mg/kg) significantly decreased this time (p < 0.01) by 64.41% and 76.27%, respectively, compared to the vehicle control. The time spent in the platform compartment was significantly (p < 0.01) decreased by 49.11% in scopolamine-treated animals compared to the normal control. Preventive treatment with EA (36 mg/kg) significantly (p < 0.001) increased this time by 110.45% compared to vehicle control. Preventive treatment with donepezil and aqueous extract (144 mg/kg) significantly increased this time (p < 0.01) by 94.03% and 89.55%, respectively, compared to the vehicle control.

3.3 Effects of the Aqueous Extract of B. atroviridis on Depression

To assess the anti-depressive effects of EA, the forced swim (FS) and open arena (OA) tests were performed. Besides, serotonin levels (5-HT) were assessed in both the cortex and hippocampus.

Effects of the aqueous extract of B. atroviridis in the Forced Swim Test (FS): Fig. 4 shows the effects of an aqueous extract of B. atroviridis (EA) on immobility, swimming, and climbing times recorded during the Forced Swim Test (FS). Immobility time was significantly (p<0.001) increased by 13.91% in the scopolamine-treated rats compared to the normal control. Administration of EA (36 mg/kg) resulted in a significant (p<0.001) decrease in immobility time of 21.59% compared to the vehicle control. Treatment with EA at 72 and 144 mg/kg resulted in a significant decrease in immobility time (p<0.001) of 19.77% and 22.92%, respectively, compared to the vehicle control. Imipramine treatment induced a significant decrease in immobility time (p<0.01) of 15.83% compared to the vehicle control. Swimming and climbing times were significantly (p < 0.01) decreased by 23.15% and 32.95%, respectively, in scopolamine-treated animals compared to the normal control. EA-treated animals (36 mg/kg) presented both high swimming and climbing times compared to the vehicle group (35.80% and 39.07%, respectively). Furthermore, EA at 72 and 144 mg/kg significantly (p < 0.001) increased the climbing time by 60.04% and 53.82%, respectively, compared to the vehicle control. Imipramine treatment induced a significant (p < 0.01) increase in swimming and climbing times of 44.71% and 30.46%, respectively, compared to the vehicle control.
Fig. 2. Effects of the aqueous extract of *B. atroviridis* on scopolamine-induced memory impairment in the Novel Object Recognition Test (NOR) (on the recognition index) Each bar represents the average ± SEM; n = 7; \(^{\text{b}} p < 0.01\) vs. normal control; \(^{\text{c}} p < 0.01\), \(^{\text{d}} p < 0.001\) vs. H\(_2\)O + Sco. H\(_2\)O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H\(_2\)O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg

Fig. 3. Effects of aqueous *B. atroviridis* extract on scopolamine-induced memory impairment in the Morris Water Maze Test (MWM) (on the latency time and the time spent in the target quadrant) Each bar represents the average ± SEM; n = 7; \(^{\text{b}} p < 0.01\), \(^{\text{c}} p < 0.001\) vs. normal control; \(^{\text{1}} p < 0.05\), \(^{\text{2}} p < 0.01\), \(^{\text{3}} p < 0.001\) vs. H\(_2\)O + Sco. H\(_2\)O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H\(_2\)O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg

Fig. 4. Effects of aqueous *B. atroviridis* extract on scopolamine-induced depression in the Forced Swim test (FS) (on immobility, swimming, and climbing times) Each bar represents the average ± SEM; n = 7; \(^{\text{b}} p < 0.01\), \(^{\text{c}} p < 0.001\) vs. normal control; \(^{\text{1}} p < 0.05\), \(^{\text{2}} p < 0.01\), \(^{\text{3}} p < 0.001\) vs. H\(_2\)O + Sco. H\(_2\)O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H\(_2\)O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg
Effects of the aqueous extract of *B. atroviridis* in the Open Arena Test (OA): Fig. 5 shows the effects of the aqueous extract of *B. atroviridis* (EA) on the number of lines crossed, rearing, and grooming during the Open Arena Test (OA). The number of lines crossed decreased significantly (p < 0.01) by 41.33% in the scopolamine-treated rats compared to the normal control. Preventive treatment with the extract at 72 and 144 mg/kg significantly (p < 0.001) increased this number by 73.91% and 90.43%, respectively, compared to the vehicle control. Aqueous extract (36 mg/kg) significantly increased this number (p < 0.05) by 56.52% compared to the vehicle control. Preventive treatment with imipramine significantly increased the number of crossings (p < 0.01) by 60.87% compared to vehicle control. The number of rearing significantly (p < 0.01) decreased by 31.58% in scopolamine-treated rats compared to the normal control. Preventive treatment with extract at 36 and 144 mg/kg significantly (p < 0.001) increased the number of rearing by 58.97% and 76.92%, respectively, compared to the vehicle control. Preventive treatment with imipramine significantly (p < 0.01) increased the number of rearing by 56.52% compared to the vehicle control.

Effects of the aqueous extract of *B. atroviridis* on serotonin (5-HT) level: Fig. 6 illustrates the effects of *B. atroviridis* aqueous extract (EA) on cortical and hippocampal serotonin (5-HT) concentrations. Scopolamine-treated rats had a low value for 5-HT levels in the cortex, 54.49% lower than the normal control. EA-treated rats at 36 mg/kg had a 212.53% increase in 5-HT level compared to the vehicle control (p < 0.001). When compared to the vehicle control, the aqueous extract at 74 and 144 mg/kg resulted in a significant (p < 0.01) increase in the 5-HT concentration of 135.43% and 121.70%, respectively. Scopolamine reduced 5-HT levels in the hippocampus by 47.25% in the negative control compared to the normal control (p < 0.05). The administration of EA at 36 mg/kg resulted in a significant (p < 0.001) increase in 5-HT by 195.94% compared to the negative control. The imipramine treatment resulted in a significant (p < 0.01) increase in 5-HT levels both in the cortex and the hippocampus compared to the vehicle control.

3.4 Effects of the Aqueous Extract of *B. atroviridis* on Episodic and Semantic Memories

Acetylcholine level (ACh) and acetylcholinesterase activity (AChase) were measured in the cortex and hippocampus to demonstrate the effects of the aqueous extract of *B. atroviridis* (EA) on episodic and semantic memories. Fig. 7 presents EA’s effects on ACh levels in the cortex and hippocampus. Scopolamine-treated rats had a significant (p < 0.001) decrease in ACh level in the cortex compared to the normal control. Furthermore, they had a higher AChase activity (64.37%) than the control group. When compared to the vehicle control, EA at 144 mg/kg resulted in a significant (p < 0.001) increase in ACh level (94%) and a decrease in AChase activity (48.68%) in the cortex. Scopolamine caused a significant (p < 0.001) decrease in ACh level (58.95%) and an increase in AChase activity (49.74%) in rats’ hippocampus when compared to the normal control. If compared to the vehicle control, EA-treated rats (144 mg/kg) had a significant (p < 0.001) increase in ACh level (120.36%) and a decrease in AChase activity (58.25%). When compared to the vehicle control, donepezil treatment resulted in a significant (p < 0.001) increase in ACh level and a decrease in AChase activity in the hippocampus.

3.5 Effects of the Aqueous *B. atroviridis* Extract on Oxidative Stress Parameters

Malondialdehyde (MDA), reduced glutathione (GSH), and nitrite levels in the cortex and hippocampus were measured to assess the antioxidant activities of *B. atroviridis*’ aqueous extract (EA). The effects of *B. atroviridis* on cortical and hippocampal MDA, GSH, and nitrite concentrations are shown in Table 1. Scopolamine-treated animals had a significant (p < 0.001) increase in cortical and hippocampal MDA and a significant (p < 0.001) decrease in cortical and hippocampal GSH and nitrite concentrations. EA at 72 and 144 mg/kg doses resulted in a significant (p < 0.001) decrease in MDA in the cortex of 43.52% and 41.62%, respectively, when compared to the vehicle control. If compared to the vehicle control, the *B. atroviridis* aqueous extract at 72 mg/kg caused a significant (p < 0.05) decrease in MDA in the hippocampus. Preventive treatment with donepezil reduced this marker in the cortex and hippocampus by 43.19% and 44.57%, respectively when compared to the negative control. EA-treated rats at 72 and 144 mg/kg showed a significant (p < 0.001) increase in GSH in the cortex by 120.28% and 155.51%, respectively, as opposed to the negative control. Once compared to the vehicle control, aqueous...
Fig. 5. Effects of aqueous *B. atroviridis* extract on scopolamine-induced depression in the Open Arena Test (OA) (on the number of lines crossed, the number of elevations, and grooming) Each bar represents the average ± SEM; n = 7; \( a \) \( p < 0.05 \), \( b \) \( p < 0.01 \), \( c \) \( p < 0.001 \) vs. normal control; \( d \) \( p < 0.05 \), \( e \) \( p < 0.01 \), \( f \) \( p < 0.001 \) vs. \( H_2O + Sco \). \( H_2O + NaCl \): distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); \( H_2O + Sco \): distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg.

Fig. 6. Effects of the aqueous extract of *B. atroviridis* on serotonin (5-HT) concentrations in the cortex and hippocampus Each bar represents the average ± SEM; n = 7; \( a \) \( p < 0.05 \), \( b \) \( p < 0.01 \), \( c \) \( p < 0.001 \) vs. normal control; \( d \) \( p < 0.05 \), \( e \) \( p < 0.01 \), \( f \) \( p < 0.001 \) vs. \( H_2O + Sco \). \( H_2O + NaCl \): distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); \( H_2O + Sco \): distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg.

Fig. 7. Effects of the aqueous extract of *B. atroviridis* on semantic and episodic memories (A: acetylcholine level in the cortex and hippocampus; B: acetylcholinesterase activity in the cortex and hippocampus) Each bar represents the average ± SEM; n = 7; \( a \) \( p < 0.05 \), \( b \) \( p < 0.01 \), \( c \) \( p < 0.001 \) vs. normal control; \( d \) \( p < 0.05 \), \( e \) \( p < 0.01 \), \( f \) \( p < 0.001 \) vs. \( H_2O + Sco \). \( H_2O + NaCl \): distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); \( H_2O + Sco \): distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg.
Table 1. Effects of aqueous extract of *B. atroviridis* on some parameters of oxidative status

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Cortex</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (µmol/g)</td>
<td>GSH (µmol/g)x10⁻²</td>
<td>Nitrites (µmol/g)x10⁻²</td>
</tr>
<tr>
<td>H₂O + NaCl</td>
<td>7.75 ± 0.92</td>
<td>92.81 ± 11.39</td>
<td>183.61 ± 5.31</td>
</tr>
<tr>
<td>H₂O + Sco</td>
<td>15.15 ± 0.68⁶</td>
<td>54.64 ± 45.44⁶</td>
<td>95.55 ± 2.40³</td>
</tr>
<tr>
<td>DNPZ + Sco</td>
<td>8.61 ± 0.86³</td>
<td>75.44 ± 93.89²</td>
<td>182.14 ± 1.11³</td>
</tr>
<tr>
<td>IPME + Sco</td>
<td>11.26 ± 1.24</td>
<td>77.73 ± 28.12²</td>
<td>184.52 ± 13.32³</td>
</tr>
<tr>
<td>EA 36 + Sco</td>
<td>11.04 ± 0.81¹</td>
<td>66.37 ± 35.29¹</td>
<td>170.54 ± 23.41²</td>
</tr>
<tr>
<td>EA 72 + Sco</td>
<td>8.56 ± 0.54³</td>
<td>80.71 ± 16.65³</td>
<td>157.9 ± 6.30¹</td>
</tr>
<tr>
<td>EA 144 + Sco</td>
<td>8.84 ± 0.97³</td>
<td>93.62 ± 32.11³</td>
<td>156.63 ± 6.11¹</td>
</tr>
</tbody>
</table>

Each value represents the average ± SEM; n = 7; "p < 0.05, "p < 0.001 vs. normal control; "p < 0.05, "p < 0.01, "p < 0.001 vs. H₂O + Sco. H₂O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H₂O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg.
extract at doses of 72 and 144 mg/kg caused a significant (p < 0.05) increase in nitrite concentration in the cortex of 65.25% and 63.89%, respectively. Preventive treatment with EA at doses of 72 and 144 mg/kg resulted in a significant (p < 0.01) increase in hippocampal nitrite concentration of 44.31% and 44.88%, respectively, as compared to the vehicle control. Preventive treatment with donepezil and imipramine resulted in a significant (p < 0.001) increase in this concentration in the cortex of 90.58% and 93.09%, respectively, if compared to the vehicle control.

### 3.6 Effect of the Aqueous Extract of B. atroviridis on Neurometabolic Activities

To assess the effects of EA treatment on neurometabolic activities, concentrations of macronutrients (calcium and magnesium) were evaluated both in the cortex and the hippocampus. Results are summarized in Fig. 8. Scopolamine caused a significant increase (p < 0.01) in calcium and a significant decrease (p < 0.001) in magnesium levels both in the cortex and the hippocampus compared to the normal control. Aqueous extract at 72 and 144 mg/kg significantly (p < 0.001) decreased calcium levels by 33.66% and 45%, respectively, compared to the vehicle control. In the hippocampus, the administration of scopolamine resulted in a significant (p < 0.01) increase in calcium level of 36.72% in the vehicle control compared to the normal control. The aqueous extract at 72 and 144 mg/kg resulted in a significant decrease in calcium concentration of 23.45% and 25.92%, respectively, if compared to the negative control. The aqueous extract at 72 and 144 mg/kg resulted in a significant increase (p < 0.01) of this macronutrient by 52.78% and (p < 0.05) by 45.84%, respectively, as compared to the negative control. In the hippocampus, scopolamine caused a significant (p < 0.001) decrease in magnesium concentration of 66.27% in the negative control compared to the normal control. The aqueous extract at 72 and 144 mg/kg resulted in a significant (p < 0.001) increase of 115.64% and 169.69%, respectively, when compared to the negative control.

### 3.7 Effects of the Aqueous Extract of B. atroviridis Extract on the Morphology and the Density of Neurons in the Cortex and CA1, CA3 Regions, and the Dentate Gyrus of the Hippocampus

To evaluate B. atroviridis’ effects on the morphology in the cortex and in CA1, CA3, and the dentate gyrus of the hippocampus, histopathological analyses were carried out using paraffin-embedded histological sections stained with Hematoxylin-Eosin. Furthermore, neuronal density was assessed in both CA1 and CA3 regions by using histomorphometry.

Fig. 8. Effect of aqueous extract of B. atroviridis on neurometabolic activities (calcium (A) and magnesium (B) concentrations in the cortex and hippocampus). Each bar represents the average ± SEM; n = 7; b p < 0.01, c p < 0.001 vs. normal control; 1 p < 0.05, 2 p < 0.01, 3 p < 0.001 vs. H₂O + Sco. H₂O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H₂O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of B. atroviridis at respective doses of 36, 72, and 144 mg/kg.
Fig. 9. Effects of aqueous *B. atroviridis* extract on the microarchitecture of the hippocampus and cortex (Hematoxylin-eosin staining, x 400) A: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); B: distilled water (10 mL/kg) and scopolamine (1 mg/kg); C: donepezil (6 mg/kg) and scopolamine (1 mg/kg); D: imipramine (12 mg/kg) and scopolamine (1 mg/kg); E, F, G: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg. Nn = normal neuron, Nv = neuronal vacuolation, Nde = neuronal degeneration, Hn = hyperchromatic nucleus, CL = chromatolysis, Nd = neuronal disorganization, NL = neuronal loss, Gc = granular cell layer, Pc = polymorphic cell layer, Mc = molecular layer, Ce = cerebral edema
Fig. 10. Effects of aqueous *B. atroviridis* extract on neuronal density in CA1 and CA3 regions of the hippocampus. Each bar represents the average ± SEM; n = 7; *p < 0.05, *p < 0.01, *p < 0.001 vs. normal control; *p < 0.05, *p < 0.01, *p < 0.001 vs. H₂O + Sco. H₂O + NaCl: distilled water (10 mL/kg) and 0.9% saline (1 mL/kg); H₂O + Sco: distilled water (10 mL/kg) and scopolamine (1 mg/kg); DNPZ + Sco: donepezil (6 mg/kg) and scopolamine (1 mg/kg); IPME + Sco: imipramine (12 mg/kg) and scopolamine (1 mg/kg); EA 36, 72, and 144: aqueous extract of *B. atroviridis* at respective doses of 36, 72, and 144 mg/kg

**Effects of the aqueous extract of *B. atroviridis* extract on neurons morphology:** Fig. 9 shows the effects of *B. atroviridis* extract on the microarchitecture of the hippocampus and cortex. Compared to normal control, scopolamine caused disorganization and neuronal loss in the CA1 and CA3 regions of the hippocampus. These areas also show hyperchromatic nuclei with chromatolysis. In the dentatus gyrus, scopolamine-treated animals showed degeneration of granular neurons compared to the normal control. The same is true of the cortex, which shows hyperchromatic nuclei, cerebral edema, and neuronal vacuolation. Treatment with donepezil, imipramine, and EA at doses of 72 and 144 mg/kg prevented these alterations.

**Effects of *B. atroviridis* aqueous extract on neuronal density in CA1 and CA3 regions of the hippocampus:** Fig. 10 shows the effects of *B. atroviridis* on the density of neurons in the CA1 and CA3 regions of the hippocampus. From this figure, it can be seen that scopolamine in the CA1 and CA3 regions significantly (p < 0.001) reduced the number of neurons in the negative control by 40.71% and 40.09%, respectively, as compared to the normal control. In the CA3 region, the plant extract at 72 and 144 mg/kg significantly (p < 0.01) increased the number of neurons by 38.58% and 44.88%, respectively, when compared to the negative control.

### 4. DISCUSSION

This study aimed to evaluate the preventive anti-amnestic and antidepressant effects of the aqueous extract of *B. atroviridis* bark on a model of AD induced by 15-day scopolamine administration in Wistar rats. The present study shows that scopolamine administration induced in rats a decrease in the recognition index in NOR, which, according to Brodziak et al., assesses short-term memory [37]. Scopolamine also increased the latency to find the target frame and reduced the time spent in the MWM, which assesses long-term memory [38,39]. Behavioral analysis shows that scopolamine-induced disturbances in both long- and short-term memory occurred after two weeks of administration. Scopolamine pathways include two mechanisms: the inhibition of cholinergic neurotransmission by antagonizing central muscarinic receptors. Moreover, it induced oxidative stress, subsequently leading to neuronal death through apoptotic pathways [39,40]. In this study, scopolamine caused a significant increase in MDA levels and a decrease in GSH and nitrite levels. Administration of *B. atroviridis*’s extract prevented disturbances in brain oxidative status by increasing GSH and nitrite levels, coupled with the inhibition of neuronal peroxidation characterized by low MDA levels. Indeed, this action of EA can be due to flavonoids, secondary metabolites with a high antioxidant power linked
to the inhibition of NADPH oxidase, which generates free radicals in the cellular mitochondria [41]. The installation of memory disorders is the result of mechanisms involving the production of free radicals and disorders of cholinergic neurotransmission [42,43]. In most cases, the production of free radicals in nerve cells involves an increased influx of Ca$^{2+}$ into neurons through the activation of N-methyl-D-aspartate (NMDA) receptors, a process responsible for excitotoxicity [44]. The results of the present work show that scopolamine disturbs neuromodulation by increasing Ca$^{2+}$ and decreasing Mg$^{2+}$ in both the hippocampus and cerebral cortex. An increase in AChase activity and a decrease in hippocampal and cortical ACh levels were also noted. Indeed, it has been shown that muscarinic M1 receptors and the NR1 subunit of NMDA receptors are co-located in hippocampal pyramidal neurons, and consequently, the administration of scopolamine in rats leads to the co-activation of M1 receptors and the NR1 subunit with consequent amplification of NMDA receptor activation [45-48]. Furthermore, it is established that reactive oxygen species stimulate the activity of Ca$^{2+}$ channels and inhibit that of calcium pumps [49]. These actions potentiate calcium influx into neurons and would be a candidate pathway by which scopolamine would induce cell damage. Fluctuating ACh and disruption of AChase activity have been shown to result in episodic and semantic memory impairment [50]. The blockade of muscarinic receptors scattered in the brain by scopolamine is, therefore, a source of dysfunctional cholinergic neurotransmission and a source of long- and short-term memory loss [51].

B. atroviridis aqueous extract prevented scopolamine-induced effects on AChase and ACh. The extract also prevented the increase in hippocampal and cortical calcium levels and the decrease in magnesium levels in both the cortex and hippocampus. This activity would be attributed to the flavonoids present in the plant, which are known to have anti-amnesic activities by inhibiting acetylcholinesterase and increasing cerebral acetylcholine levels. Besides, the antioxidant power of the plant would also inhibit ROS-induced calcium influx and overall maintain the homeostasis of the macronutrients [52,53]. Thus, EA prevented the memory impairment induced by scopolamine in rats.

Depression can be a comorbidity of memory disorders, especially in Alzheimer’s disease [54]. The OA and FS tests are good tests for evaluating the antidepressant potential of many molecules. The results of the present work show that scopolamine significantly reduced swimming time and increased immobility time in the forced swimming test. Similarly, there was a decrease in the number of lines crossed and the number of times the animal stood on two legs in OA. These results indicate a state of depression. This result is similar to that of Humna et al. [55] who reported an installation of depression in rats rendered amnesic by a scopolamine injection. The same was true for 5-HT, which was significantly decreased in rats receiving scopolamine. Serotonin is a neurotransmitter known for its anxiolytic and antidepressant potential. The decrease in its concentration caused by scopolamine could be a cause of depression. The extract prevented depression by decreasing the production of 5-HT. This activity would be attributed to flavonoids, which are known to inhibit monoamine oxidase degradation. Behavioral dysfunctions (memory loss and depression) are reflected histologically by neurofibrillary degeneration of the hippocampus, with a decrease in the number of neurons in the CA1 and CA2 regions [56-59]. Similar results were obtained by Baek et al. [60] in a scopolamine-induced amnesia model. These hippocampal alterations are thought to be a consequence of the deleterious activity of free radicals at the brain level. According to Samina [61], oxidative stress-induced damage plays an important role in the pathogenesis of many central nervous system disorders. Indeed, reactive oxygen species are known to induce massive degeneration of hippocampal neurons [62] through the induction of lipid peroxidation of cell membranes. The extract prevented this nerve degeneration, which could be explained by its antioxidant activities. Soursop fruit extract and Bergapten, as well as B. atroviridis, show both antioxidative and anti-amnesic activities in a scopolamine-induced amnesia model [63,64].

5. CONCLUSION

Globally, Bridelia atroviridis aqueous extract (EA) significantly prevented scopolamine-induced memory impairment and depression in rats. The extract possesses antidepressant and anti-amnesic effects by improving cholinergic and serotoninergic modulations, hippocampal-dependent cognition, oxidative status, and neurodegeneration. This study offers EA at a dose of 144 mg/kg which suggests the possibility of its development as a healthy functional food for the improvement of depression and memory impairment.
DATA ACCESSIBILITY STATEMENT

All data are provided in full in the results section of this paper.

DISCLOSURE INSTRUCTIONS

During the preparation of this work, the author(s) used QuillBot (Course Hero), LLC. 2023, to check the grammar and rephrase sentences of the methodology. After using this service, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were carried out following the principles and procedures of the European Union on Animal Care (CEE Council 86/609) as adopted by the Cameroon Institutional National Ethic Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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