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Khaya grandifololia Improves Cognition and Prevents Scopolamine-Induced Impairment of Brain Functions by Activating the Cholinergic and Antioxidant Systems in Rats

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Abstract: Alzheimer's disease (AD) is a multifactorial, progressive neurodegenerative disorder with dementia and persistent impairment of cognitive functions as the main clinical characteristics. Although signs of progress are being made in developing AD therapy, there is no effective drug capable of stopping and/or slowing down the progression of the disease. We have previously indicated in an in vitro setting of AD that *Khaya grandifololia* (KG) crude extract possesses antioxidant, anti-inflammatory, and neuroprotective activities. In the current work, we have evaluated the activity of KG hydroethanolic (KG-HE) extract in preventing cognitive impairment and promoting memory improvement in vivo. Results from behavioral tests indicated a significant improvement in memory performance and a delay in depression-like behavior upon treatment of rats with KG-HE extract (5, 25, and 50 mg/kg) or donepezil (1 mg/kg) as standard. Because scopolamine (1 mg/kg) impaired cognitive performance in the tail suspension test, Morris Water Maze test, and Novelty Suppressed Feeding Test, KG-HE extract (5, 25, and 50 mg/kg) or donepezil (1 mg/kg) treatment prevented scopolamine-induced performance impairment. Moreover, both KG-HE extract (5, 25, and 50 mg/kg) and donepezil (1 mg/kg) prevented the scopolamine-induced cognitive impairment by inhibiting the acetylcholinesterase activity. In addition, the brain parameters of stress oxidation (SOD, CAT, and GSH) reduced by scopolamine treatment were regulated by the administration of KG-HE extract or the standard drug donepezil. An increase in the MDA level and the phosphatase activity both in the serum and brain due to scopolamine treatment was restored by the administration of KG-HE extract or donepezil. Taken together, these results suggest that KG-HE extract improves cognition and relieves the scopolamine-induced cognitive impairment via activation of the cholinergic and anti-oxidation systems in rats.

Keywords: *Khaya grandifololia* Hydroethanolic Extract, Alzheimer Disease, Cognition, Neuroprotection, Anxiety, Depression, Cholinergic System, Stress Oxidation

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects cognitive functions and is clinically characterized by behavioral deterioration, performance impairment, severe depression, loss of autonomy, or death of the patient [1, 2]. Biochemically, the disease is characterized by a beta-amyloid deposit in the brain. It is widely recognized that senile plaques are polymorphous beta-amyloid protein deposits

found in the brain in Alzheimer's disease and normal aging brain [3]. Furthermore, among the two existing amyloid proteins, beta-amyloid 42 is found in senile plaque, whereas beta-amyloid 40 is mostly found in physiological fluids [3, 4]. In addition to amyloid protein deposits, the hyper-phosphorylation of tau proteins is another biochemical characteristic of Alzheimer's disease and neurodegeneration [3]. In Alzheimer's disease, the beta-amyloid deposits and tau hyperphosphorylation are biochemical changes that lead to clinical characteristics such as behavioral changes and cognitive impairment [1].

Alzheimer's disease is the first cause of degenerative dementia and affects 60 to 70% of people living with dementia worldwide [4, 5]. In the course of AD pathology, neural and cognitive dysfunctions appear because of the oxidative damage to nucleic acids, proteins, and mitochondria sustained by the brain cells. Epidemiological studies have indicated that the number of people suffering from dementia will increase from 57.4 million to 152.8 million by 2050. In Africa, there are nearly 3.5 million cases of dementia [6, 7], and in Cameroon, there is a prevalence of 3.9% [8].

The management of AD relies on various allopathic drugs that induce side effects such as severe psychological stress or amnesia. Therefore, alternative medicine using medicinal plants could be a great source of drugs for the treatment of AD and memory dysfunction with fewer or no side effects. Medicinal plants are an interesting source in the search for bioactive molecules with neuroprotective properties [9]. An increasing number of investigations have shown that some plant species exhibit a variety of promising pharmacological properties, including antioxidant, anti-anxiolytic, and antidepressant effects [10, 11].

Medicinal plants have been screened for therapeutic compounds possessing anti-amyloidogenic properties [12, 13], inhibitors of acetylcholine esterase activity [14], Aβ peptide fibrillation inhibitors [15], anti-inflammatory properties [16], secretase inhibitors [17], anti-aging and antiamnesic potential [18]. Moreover, *Khaya grandifolia*, a plant of the family of Meliaceae, is used in traditional medicine to treat malaria, cancer, feverish illnesses, ulcers, and convulsions [19]. Previous work carried out on KG showed that it has immunomodulatory, anti-inflammatory [20], antioxidant [21], cytoprotective [22], and hepatoprotective [23] effects. We have recently shown that KG extract restores mitochondrial function, inhibits apoptosis, synaptic toxicity, and hyperphosphorylation of tau protein [24]. In the current study, we used an *in vivo* rat model of Alzheimer's to demonstrate the prevention of memory dysfunction and the antioxidant and antianxiety properties of KG hydroethanolic extract.

2. Materials and Methods

2.1. Biological Materials and Chemicals

Khaya grandifolia (KG) (Cameroon National Herbarium reference number 23434 YA) stem bark was collected in Foumban, the head of the Noun division of the West region in Cameroon. Male Albinos Wistar rats, aged 8 to 10 weeks old and weighting between 250 to 300 g were bred and housed in the animal facility of the department of biochemistry, University of Yaoundé 1 according to the Guide for the *NIH-Care and Use of Laboratory Animals (8th Edition)*. Scopolamine was purchased from Cooper (Copper, France). Donepezil was from Aricept (Aricept, France). Acetylthiocholine iodide, 5,5-dithiobis (2-nitro-benzoic acid), trichloroacetic acid, thiobarbituric acid, para nitrophenylphosphate, Congo red were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of the Hydro-ethanolic Extract of Khaya grandifololia (KG-HE)

Fresh KG stem barks were washed with distilled water, air-dried, and powdered. At room temperature, 200 g of the powder was extracted twice with 2 L of ethanol/water 65/35 (v/v) with regular agitation for 48 hours. The filtered solutions were pooled and evaporated to dryness using a rotary evaporator before drying in an oven (HP-AD070, Memmert; Germany) at 50°C.

2.3. Animal and Administration of the Hydro-ethanolic Extract of Khaya grandifololia (KG)

Albino Adult rats (n = 25), weighing 250 to 300 g at the beginning of the experiment, were housed in the animal facility of the department of biochemistry, University of Yaoundé 1, according to the Guide for the *NIH-Care and Use of Laboratory Animals (8th Edition)*. All rats used in this study were kept on a 12/12 light/dark cycle and a constant ambient temperature of 25± 1°C. All animals were provided a standard rat diet and water *ad libitum*. All experiments conducted in this study were approved by the Ethics Committee of the Faculty of Sciences of the University of Yaoundé 1 (permit number: 22/0415/UY1/D/FS/VD-RC).

For the memory improvement (Nootropic) model investigation, rats were randomly divided into five groups, with five rats per group: Rats in the control group (1) received saline solution (10 ml/kg); rats in the group (2), (3), and (4) were administered the KG-HE extract at 5, 25, and 50 mg/kg, respectively; rats in the group (5) were administered donepezil (1 mg/kg). Pretreatment of all groups with drugs or KG-HE extract lasted 6 consecutive days before the rats were subjected to behavioral tests on day 7 for the tail suspension test (TST) and day 8 for the Morris water maze (MWM) (Figure 1).

For the scopolamine activity model, rats were randomly divided into six groups: rats in group (1) (normal control) were administered methyl cellulose (0.5%); rats in group (2) (model group) received scopolamine (1 mg/kg, *i.p.*); rats in group (3) (positive control) received scopolamine (1 mg/kg, *i.p.*) and donepezil (1 mg/kg, *p.o.*); rats in group (4), (5) and (6) were treated with scopolamine (1 mg/kg, *i.p.*) together with KG-HE extract at 5, 25, and 50 mg/kg, respectively. The volume of solution administered to each rat was 10 ml/kg.

The KG-HE extract or donepezil was administered once per day throughout the experimental period (14 days). Scopolamine was administered every day from the eighth day to the end of the experiment (Figure 1). The cognitive and memory functions of mice were assessed 30 min after the administration of scopolamine.

For the scopolamine model, amnesia was induced in all the groups except the control group by daily intraperitoneal injections of scopolamine (1 mg/kg) for 9 days after KG-HE extract pre-treatment (Day 9 to Day 17). Thirty minutes prior to the administration of scopolamine, TST was conducted on day 14, MWM was carried out on days 15, and the novelty-suppressed feeding test (NSFT) on day 17 (Figure 1).

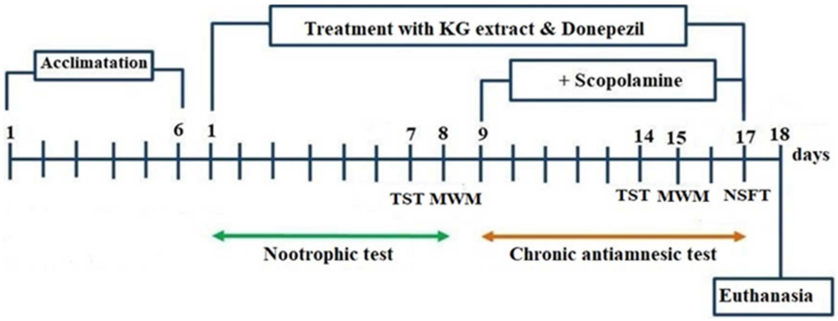

Figure 1. *Experimental design.*

2.4. Behavioral Analysis

2.4.1. Tail Suspension Test (TST)

The tail suspension test (TST) was performed according to the method described by Steru and colleagues [25]. Briefly, following treatment with drugs (donepezil, scopolamine, and/or KG-HE extract, rats were suspended on the edge of the table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded for the last 4 min during the 6-min period. Rats were considered immobile when they hung passively and completely motionless.

2.4.2. Morris Water Maze (MWM)

A test was conducted to evaluate the therapeutic effect of the drugs and KG-HE extract for spatial reference learning and memory in a water maze. The water maze consists of a large circular pool filled to a depth of 30 cm with water at 20 ± 2°C. A platform was placed inside the pool, 0.5 cm below the surface of the water. The position of the platform was fixed, and the rats, facing the pool wall, were thrown into the water, freely swimming for 60 s. If the rats failed to find the platform within 60 s, the training ended and then the rats were guided to the platform and kept on it for 10 s. The rats entered the water training twice every day, keeping the position of the platform and surrounding objects constant, and were trained continuously for 4 days. The orientation navigation test was conducted on the day of the experiment for 60 s, and the time taken for the rats to reach the platform was recorded. Those who could not find the platform were counted as 60 s. The latency to reach the platform was recorded by a camera, mounted above the center of the water maze [26].

2.4.3. Novelty-Suppressed Feeding Test (NSFT)

The novelty-suppressed feeding test (NSF) was used to measure depression-like behaviors. This test was performed by scoring the latency to feed for food-deprived rats when they were introduced to an unfamiliar environment. Rats were food-deprived for 24 hours prior to the test, with free access to water, and were moved to the dimly lit testing room one to two hours before the test. Rats were placed in one corner of an open field apparatus (17 inch × 17 inch × 12 inch) with clear acrylic walls and an opaque white acrylic floor. The light intensity in the open field was maintained at 16-20 Lux and the walls and floor were wiped with Novalsan (chlorhexidine diacetate) (Zeotis, Tampa, Florida, USA) between trials. A food pellet was placed in the center of the open field, and rats were placed in one corner. Feeding behavior was observed by an experimenter blind to the rat group. Latencies to approach and to begin eating were recorded, with a limit of 5 minutes. As soon as the rat was observed to eat, or the 5-minute time limit was reached, the rat was removed from the open field and placed in the home cage and observed until it began to eat [27].

2.5. Tissue Preparation

After behavioral tests, the rats fasted for 12 hours before being sacrificed. After washing the dissecting instruments in 5% nitric acid and rinsing several times with distilled water, rat brains were quickly removed from the cranial box and placed on a glass support on ice or stored at -20°C for biochemistry and histology. The blood was also collected during the decapitation of the rats and immediately centrifuged at 4000 rpm for 10 minutes at 4°C, and the serum was collected and stored at -20°C until used for the analysis of biochemical parameters.

2.6. Preparation of Tissue Homogenates

After the behavioral tests, all rats were deeply anesthetized using ether. Anesthetized animals were decapitated, and the brains were rapidly removed on ice, weighed, and homogenized in cold 1.15% KCl. The homogeneity was centrifuged, at 8000 g for 15 minutes, and the supernatant was collected for protein quantitation [28] and biochemical assays.

2.7. Biochemical Evaluation

2.7.1. Determination of Superoxide Dismutase (SOD) Activity

SOD activity in rat brain homogenate was assayed by monitoring the photochemical inhibition of the nitroblue tetrazolium (NBT) reduction rate in the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide system assessed on the spectrophotometer at 540 nm [29]. The enzyme activity is expressed as units/g of the brain. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as previously described.

2.7.2. Determination of Catalase (CAT) Activity

The CAT activity in the rat brain homogenate was determined by the hydrogen peroxide reaction with ammonium molybdate, which produces a complex that absorbs at 410 nm. Briefly, 100 µL of the brain homogenate was mixed with the phosphate buffer (0.01 M, pH 7.0), followed by addition of 250 µL of H_2O_2 (0.16 M). The resultant reaction mixture was then incubated for 1 minute at 37°C, and the assay was completed by adding 1 ml of dichromate: acetic acid. A green color was formed after heating for 15 minutes, and the absorbance was obtained using the spectrophotometer at 570 nm [30]. CAT activity is expressed as mM of H_2O_2 consumed/min/g of brain.

2.7.3. Estimation of the Total Brain Glutathione (GSH) Content

Glutathione (GSH) was measured using the method of Smith and colleague [31]. DTNB (0.04%) was added to 1.1 mL of 0.25 M sodium phosphate buffer (pH 7.4) followed by the addition of 200 µL of the brain homogenate. The mixture was brought to a final volume of 1.5 mL with distilled water and the absorbance was determined using a spectrophotometer at 412 nm. GSH concentrations were expressed in micrograms of GSH/g of the brain.

2.7.4. Determination of the Level of Malondialdehyde (MDA) in Brain Homogenate and Serum

The brain and serum MDA concentrations were determined as a measure of lipid peroxidation according to the method of Wills [32]. Briefly, 2 mL of 10% brain homogenate was added to 2 mL of 10% trichloroacetic acid (TCA), and incubated at 25°C for 15 minutes. The mixture was then centrifuged, and 2 mL of TBA was added to the supernatant, heated for 10 minutes, and immediately cooled on ice for 3-5 minutes, and the absorbance was measured at 532 nm. The MDA concentration was expressed as mmol/g of the brain.

2.7.5. Acetylcholinesterase (AChE) Inhibition Assay

AChE activity was estimated in the whole brain homogenate according to the method developed by Ellman and colleagues [33]. Briefly, the brain homogenate was added to 0.1 ml of 5, 5-dithiobis (2-nitrobenzoate) (DTNB) in phosphate buffer and incubated at room temperature for 5 minutes. In addition, 0.1 ml of a freshly prepared acetylcholine iodide (pH 8) solution was added, and the change in absorbance was recorded at 412 nm on the spectrophotometer (Thermo Fischer Scientific, Waltham, Massachusetts, USA). The enzyme activity was calculated using the extinction coefficient of 14,150 $M^{-1}cm^{-1}$.

2.7.6. Phosphatase Activity

The phosphatase activity was estimated in brain homogenate and serum according to the method described by McAvoy and Nairn [34]. Briefly, 50 µL of brain homogenate or serum was diluted in Tris buffer (20 mM, pH 7.5; containing 5 mM $MgCl_2$, 1 mM EGTA, 0.02% β-mercaptoethanol and BSA 0.1 mg/ml), added to 50 µL of 10 mM p-nitrophenyl phosphate (pNPP) (dissolved in 20 mM Tris, pH 7.5), and incubated for 45 minutes at room temperature. The reaction was terminated by adding 20 µL of 5 N NaOH and the absorbance was recorded at 405 nm on a Multiskan (Thermo Fischer Scientific, Waltham, Massachusetts, USA) plate reader. KCl solution (1.15%) or distilled water was used as a blank. The activity was calculated using the following formula: activity [µmoles/min µg] = 50 [vol] × OD405nm × 1/time [min] × enzyme [µg] × 1/18,000 [molar extinction coefficient].

2.7.7. Assay for Congo Red Binding

The amyloid fibrillation was measured by the Congo red binding assay. The Congo red stock solution (5 mM) was prepared in 5 mM KH_2PO_4 buffer, pH 7.4 containing 150 mM NaCl. 200 µL of 10% brain homogenate samples treated with or without KG-HE extract were mixed with 4 mL of Congo red (5 µM). The mixture was incubated for 30 minutes at room temperature and the optical density was read at 480 nm and at 540 nm using a spectrophotometer (Thermo Fisher Scientific) against the blank [35]. The Congo binding activity (Cb) [µM] were calculated using the following formula: Cb = [(OD at 540 nm/25,295) - (OD at 480 nm/46,306)] x 1000,000].

2.8. Phytochemical Evaluation

2.8.1. Determination of Alkaloids

In 1 mL of KG-HE extract, some drops of 2% H_2SO_4 were added. In addition, some drops of Meyer's reagent were added to the mixture. The white precipitate obtained confirms the presence of alkaloids.

2.8.2. Determination of Tannin

The KG-HE extract (1.5 mL) was added to 2.5 mL of 1.5% DMSO solution. The mixture was heated at 70°C for 3 minutes and filtered. To 1.5 mL of filtrate was added 1 mL of 3% $FeCl_3$ solution. The dark green coloration in the tube clearly indicated the presence of catechotannins.

2.8.3. Flavonoid Determination

KG-HE extract (500 µL) was added to 1 mL of 1 N NaOH. An intense yellow color was produced in the plant extract, which became colorless with the addition of a few drops of diluted hydrochloric acid, and that indicates the presence of flavonoids.

2.8.4. Determination of Anthraquinone

KG-HE extract (500 µL) was mixed to 5 mL of diethyl ether and the mixture was homogenized. A 10% ammonia solution was added, and the appearance of a red or violet coloration indicated the presence of anthraquinones.

2.9. Statistical Analysis

All quantifications and enzyme activity measurements were performed in triplicates and the data are expressed as mean ± standard deviation (SD). Statistical differences were evaluated using GraphPad Prism software version 5 (San Diego, CA, USA). The comparison between groups was conducted by one–way analysis of variance (ANOVA) followed by the Tukey Multiple Comparisons Test. The results were considered statistically significant at a confidence limit of $p < 0.05$.

3. Results

3.1. Characterization of the Hydroethanolic Extract from Khaya grandifololia

The phytochemical screening of secondary metabolite content of the KG-HE extract was performed using various qualitative tests. The results obtained showed that the hydroethanolic extract of *Khaya grandifololia* was highly enriched in alkaloids, tannins, flavonoids, and anthraquinones.

3.2. Improvement of Cognitive Functions by Increasing the Morris Water Maze and Tail Suspension Scores in Khaya grandifololia-Treated Rats

The impact of KG-HE extract on memory functioning was assessed after 7 or 8 days of treatment of rats with KG-HE extract (5, 25, and 50 mg/kg) or donepezil (1 mg/kg) and the measurement of rat's scores in the Morris water maze test (Table 1). The results show that rats given donepezil- and KG-HE extracts took less time to reach the platform than control rats. In addition, rats treated with KG-HE extract spent the least amount of time reaching the platform (12.00 ± 0.67 seconds) (table 1). Moreover, a tail-suspension test was also performed in order to monitor the impact of KG-HE extract on depression-like behavior. The results show that control rats had a longer period of immobility than KG-HE-treated and donepezil-treated rats. The lowest immobility time (112.67 ± 12.22 seconds) for KG-treated rats was obtained at a concentration of 25 mg/kg. Taken together, these results indicate that the KG hydroethanolic extract may improve cognitive functions.

Table 1. *Memory improvement (nootropic) effects of Khaya grandifololia hydro-ethanolic (KG-HE) extract. Rats were orally administered the KG-HE extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.) for 8 days. The behavioral tests (tail suspension test, Morris water maze, and novelty-suppressed feeding test) were then performed on days 7th and 8th. Results are presented ± standard error of the mean for each group. The significance between rats in the control group and rats treated with KG hydroethanolic extract or donepezil was calculated using a one-way ANOVA test with post-test (Tukey's tests) [a]p<0,001 vs Control. KG 5: Khaya grandifololia 5 mg/kg; KG 25: Khaya grandifololia 25 mg/kg; KG 50: Khaya grandifololia 50 mg/kg; Don: donepezil 1 mg/kg.*

Groups		Tail suspension test (Depression-like behavior)	Morris water maze (Memory)
		Immobility time (s)	Latency time (s) to reach the platform
Group 1	Control	166,67 ± 16,44	55,67 ± 5,78
Group 2	KG 5 mg/kg	129,67 ± 15,11[a]	37,25 ± 1,25[a]
Group 3	KG 25 mg/kg	112,67 ± 12,22[a]	12,00 ± 0,67[a]
Group 4	KG 50 mg/kg	121,00 ± 9,00[a]	22,33 ± 1,78[a]
Group 5	Donepezil (1 mg/kg)	101,33 ± 7,11[a]	21,00 ± 3,56[a]

Table 2. *Evaluation of the potential of Khaya grandifololia hydroethanolic (KG-HE) extracts in preventing the scopolamine-induced brain impairment in rats. Rats were orally administered the KG hydro-ethanolic extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.) in association with scopolamine (1 mg/kg, i.p.) for 9 days. On the 14th, 15th, and 17th days, the behavioral tests (tail suspension test, Morris water maze, and novelty-suppressed feeding test) were performed. Results are presented as ± standard error of the mean for each group. The significance between rats treated with scopolamine alone and rats co-treated with KG hydroethanolic extract or donepezil in association with scopolamine was calculated using a one-way ANOVA test with post-test (Tukey's multiple comparisons) ap<0,001 vs control; bp<0,001 vs scopolamine treated group. Scop: 1 mg/kg scopolamine; KG 5: 5 mg/kg khaya grandifololia; KG 25: 25 mg/kg khaya grandifololia; KG 50: 50 mg/kg khaya grandifololia; Don: 1 mg/kg donepezil.*

Groups		Depression	Memory	Anxiety
		Immobility time (s)	Latency time (s)	Latency time (s)
Group 1	Control	101,75 ± 6,75	17,75 ± 6,12	221,00 ± 42,00
Group 2	Scopolamine (Scop)	125,20 ± 6,64	33,00 ± 4,00	296,67 ± 4,44
Group 3	KG 5 + Scop	115,80 ± 5,68	19,00 ± 6,50[a]	265,00 ± 35,00
Group 4	KG 25 + Scop	76,50 ± 7,00[a,b]	7,33 ± 0,88[b]	108,00 ± 18,67[a]
Group 5	KG 50 + Scop	78,83 ± 6,88[a,b]	8,33 ± 1,11[b]	212,33 ± 60,22
Group 6	Don + Scop	54,00 ± 6,33[a,b]	8,66 ± 0,44[b]	138,00 ± 2,00

3.3. The Hydroethanolic Extract of Khaya grandifololia Reverses Scopolamine-Induced Cognitive Impairment

To test the prevention of cognitive impairment by the KG-HE extract, the scopolamine model was used. Data analyses revealed that rats treated with scopolamine (1 mg/kg) (33.00 ± 4,00; $p<0.001$) showed a significant memory impairment when compared with control (17.75 ± 6.12) (table 2) using the Morris water maze test. In contrast, memory impairment

was not observed when rats were treated with scopolamine in association with KG-HE extracts (5, 25, and 50 mg/kg). Memory restoration was also observed when rats were treated with scopolamine (1 mg/kg) in association with donepezil (1 mg/kg), a drug known to improve cognitive functions. Likewise, depression-like behavior was induced in rats upon exposure to scopolamine (1 mg/kg) and this parameter was assessed by measuring the rat's immobility time in a tail suspension test. Results indicate that the depression-like behavior was more evident in scopolamine-treated rats, which showed a longer immobility time (125.20 ± 6.64) when compared to control rats (101.75 ± 6.75; $p<0.001$) (Table 2). Treatment of rats with scopolamine (1 mg/kg) in combination with KG-HE extracts (5, 23, and 50 mg/kg) or donepezil (1 mg/kg) led to the prevention of the depression-like behavior induced by scopolamine. The lowest immobility time was obtained with rats treated with donepezil (54.00 ± 6.33), while the lowest immobility time (76.50 ± 7,00) in rats treated with KG-HE extract was observed at a concentration of 25 mg/kg (Table 2).

We also hypothesized that the KG-HE extract might prevent the scopolamine-induced amnesia. To test this hypothesis, scopolamine alone or in association with KG-HE extracts (5.25 and 50 mg/kg) (Table 2). In control experiments, rats were either untreated or treated with scopolamine in association with donepezil (1 mg/kg), a drug known to improve memory. Results revealed that latency time (an indication of anxiety in the current experimental setting) was significantly increased in rats treated with scopolamine (1 mg/kg) when compared to untreated rats (Table 2). In contrast, treatment of rats with scopolamine (1 mg/kg) in association with KG-HE extracts (5.25 and 50 mg/kg) or donepezil (1 mg/kg), significantly reduced the anxiety-like behavior induced by scopolamine. Moreover, a more detailed analysis indicated that the highest antianxiety action of KG-HE extract was obtained with a concentration of 25 mg/kg ($p<0.0001$) (Table 2). Taken together, these results indicate that the KG-HE extract prevents scopolamine-induced cognitive impairment in rats.

3.4. The Effect of the Khaya grandifololia Hydro-ethanolic (KG-HE) Extract on Stress Oxidation Induced by Scopolamine Treatment in Rats

Stress oxidation is one of the mechanisms by which cell damage arises in neurodegenerative disorders, including Alzheimer's disease (AD). To determine whether the KG-HE extract can protect neuronal cells from a scopolamine-induced impairment, parameters of stress oxidation such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured. As shown in figure 2A, treatment of adult rats with scopolamine alone caused a significant decrease in SOD ($p<0.01$) activity. In contrast, the effect of scopolamine on SOD activity returned to normal when rats were treated with scopolamine in association with KG-HE (5 mg/kg; 25 mg/kg, 50 mg/kg) or donepezil (1 mg/kg). Similarly, CAT activity ($p<0.0001$) was significantly reduced in scopolamine-treated rats, but returned to normal when scopolamine was combined with KG-HE extract or donepezil. Only when rats were given the KG-HE extract at a concentration of 25 mg/kg ($p<0.01$) was a significant restoration of CAT activity observed (Figure 2B).

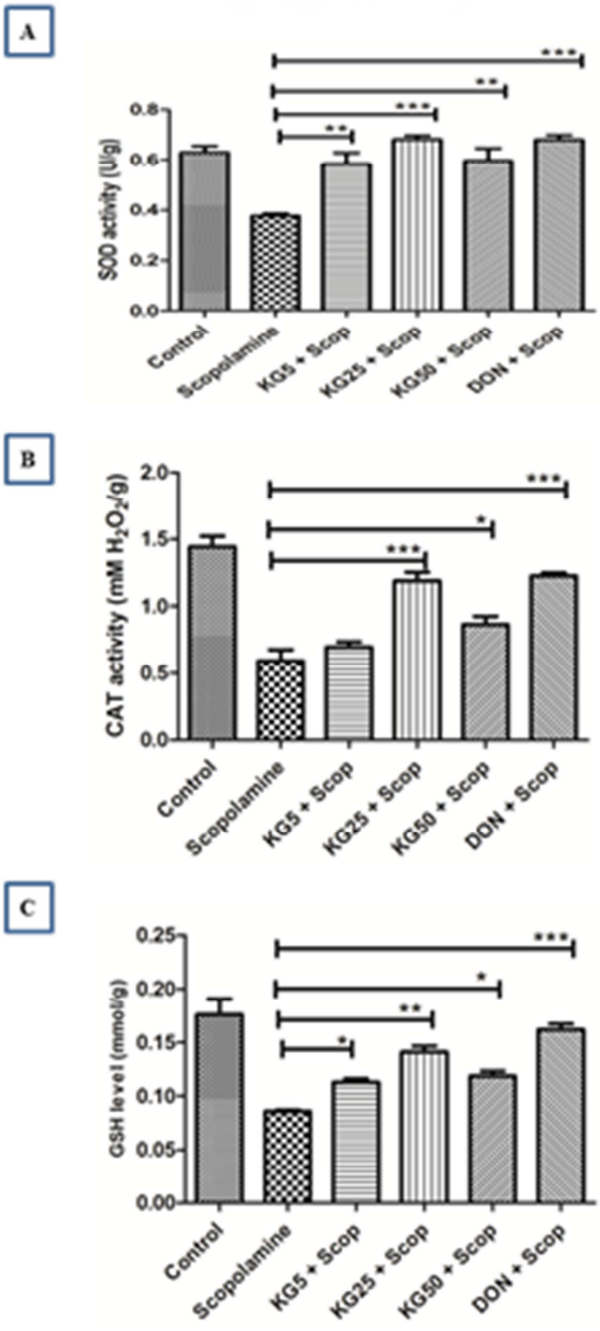

Figure 2. *Evaluation of the potential of the Khaya grandifololia hydroethanolic (KG-HE) extracts in the regulation of oxidative stress in rats treated with scopolamine. Rats were administered the solution of scopolamine (1 mg/kg, i.p.) alone or in association with KG-HE extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.). Nine (9) days following treatment, brain homogenate was prepared and used for the evaluation of the activities of the antioxidant enzymes (A) SOD (U/g/min) and (B) CAT (mM H_2O_2/g/min) and the level of reduced glutathione (C) GSH (mmol/g). Results are presented as mean ± mean standard error over animals in each group. The significance between rats treated with scopolamine alone and co-treated with scopolamine in association with KG-HE extract or donepezil is calculated by the ANOVA test (Tukey's test) *p<0.05; **p<0.001.*

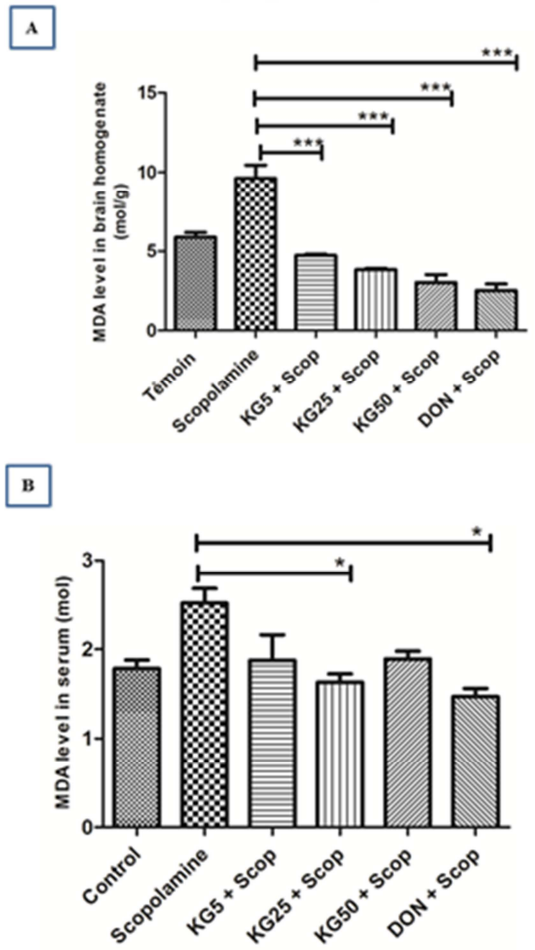

Figure 3. *Evaluation of the potential of Khaya grandifololia hydroethanolic (KG-HE) extract in the prevention of scopolamine-induced lipid peroxidation in rats. Rats were administered the solution of scopolamine (1 mg/kg, i.p.) alone or in association with KG-HE extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.). Nine (9) days following treatment, brain homogenate (A) and serum (B) were prepared and used for the evaluation of the MDA content. Results are presented as mean ± mean standard error over animals in each group. The significance between rats treated with scopolamine alone and co-treated with KG-HE extract or donepezil in combination with scopolamine is calculated by the ANOVA test (Tukey's test) *p<0.05; **p<0.001.*

To further evaluate the potential of KG-HE extract to prevent stress oxidation in a rat model of scopolamine-induced cognitive impairment, we measured the level of GSH in the brain homogenate. The results obtained indicated a significant reduction in GSH level (p<0.0001) in the brain homogenate of rats treated with scopolamine alone compared to control. In contrast, rats treated with scopolamine combined to KG-HE extract showed a restoration of the GSH level (Figure 2C). Relative GSH restoration was observed in rats treated with scopolamine in combination with KG-HE extracts at a concentration of 5 and 50 mg/kg, while significant GSH restoration was observed in rat's brains treated with scopolamine in combination with KG-HE extract at 25 mg/kg (Figure 2C). In addition, the treatment of rats with scopolamine plus donepezil (a drug known to have memory-improving activity) also showed a significant restoration in the level of GSH (Figure 2C).

We also assessed the level of malondialdehyde (MDA) as a marker for lipid peroxidation, both in the serum and brain samples from treated and untreated rats. The results showed that the MDA levels were significantly increased in scopolamine-treated rats both in the brain (Figure 3A) and the serum (Figure 3B). In contrast, rats treated with scopolamine in association with KG-HE extract or donepezil (1 mg/kg) showed a significant restoration in MDA level (p<0.001) in the brain (Figure 3A). Also, only a mild decrease in MDA levels (p<0.05) was observed in serum samples when rats were given a 25 mg/kg KG-HE extract in combination with scopolamine (Figure 3B). These observations indicated that 25 mg/kg was the KG-HE concentration capable of preventing the stress oxidation-induced damage in the rat brain.

3.5. The Khaya grandifololia Hydro-ethanolic (KG-HE) Extract Protects Brains from Scopolamine-Induced Damage by Modulating the Cholinergic System

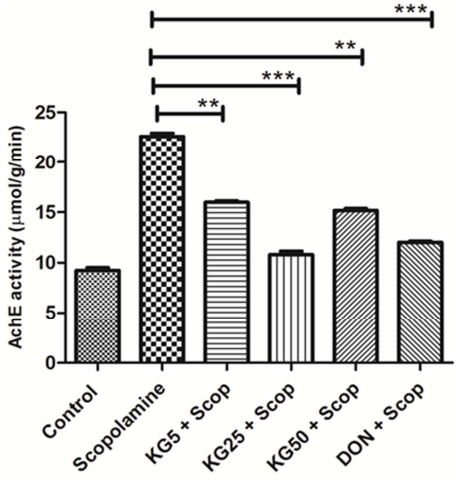

Figure 4. *Effect of the Khaya grandifololia hydroethanolic (KG-HE) extract on the cholinergic system in rats stressed with scopolamine. Rats were administered the solution of scopolamine (1 mg/kg, i.p.) alone or in association with donepezil (1 mg/kg, i.p.) or KG-HE extract (5, 25, and 50 mg/kg, p.o.). Nine (9) days following the treatment, the brain homogenates were prepared and used for the evaluation of the acetylcholinesterase activity. Results are presented as mean ± mean standard error over animals in each group. The significance between rats treated with scopolamine alone or co-treated with KG-HE extract or donepezil is calculated by the ANOVA test (Tukey's test) *p<0.05; **p<0.001.*

3.6. The Khaya grandifololia Hydro-ethanolic (KG-HE) Extract Modulates the Phosphatases Activity in Scopolamine Treated Rats

To evaluate the impact of KG-HE on the modulation of the cholinergic system in rats stressed with scopolamine, we measured the acetylcholinesterase (AChE) activity. The data in figure 4 indicate that the AChE activity in the brains of rats treated with scopolamine alone was significantly increased when compared to control rats (p<0.001). Moreover, a restoration of the AChE activity was observed in rats treated with scopolamine in association with KG-HE extract. Further examination revealed that rats treated with KG-HE extract at a concentration of 25 mg/kg showed a reduction of AChE activity to the level of control rats. This indicates that KGHE

extract administered at a concentration of 25 mg/kg is efficient in the prevention of scopolamine-induced brain damage by modulating AChE activity. We also observed a significant restoration in the AChE activity to that of the control rats when rats were treated with scopolamine in association with donepezil, a drug known to protect the brain from scopolamine-induced impairment. This data suggests that KG-HE extract may be considered a potential anticholinergic agent.

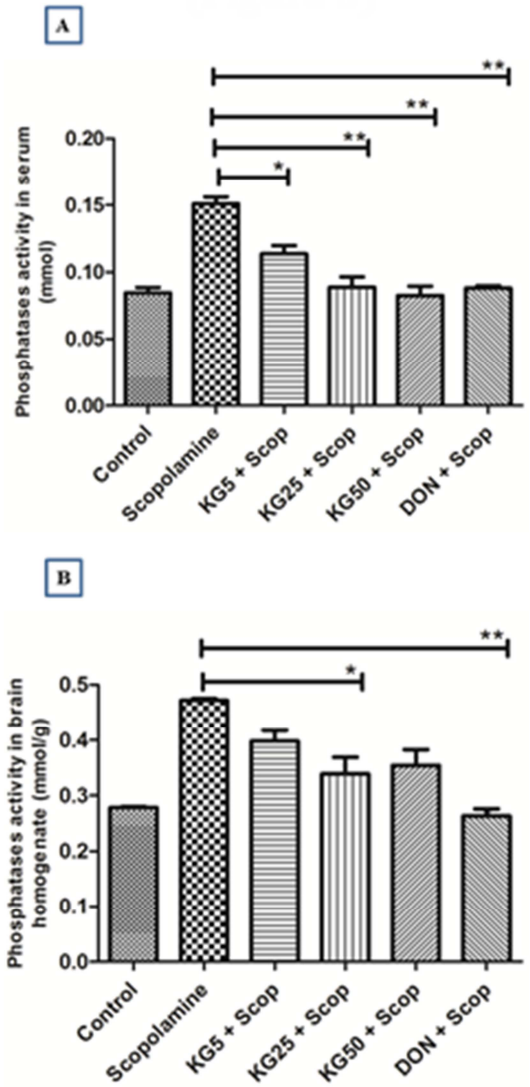

Figure 5. *Effect of the potential of Khaya grandifolia hydroethanolic (KG-HE) extract on the modulation of the phosphatase activity in rats stressed with scopolamine. Rats were administered the solution of scopolamine (1 mg/kg, i.p.) alone or in association with the KG-HE extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.). Nine (9) days following treatment, serum (A) and brain homogenate (B) were prepared and used for the evaluation of the phosphatase activity. Results are presented as mean ± mean standard error over animals in each group. The significance between rats treated with scopolamine alone and co-treated with KG hydro-ethanolic extract or donepezil in association with scopolamine is calculated by the ANOVA test (Tukey's test) *p<0.05; **p<0.001.*

To evaluate the impact of the KG-HE extract on the activity of phosphatases in rats stressed with scopolamine, we measured the phosphatase activity both in the serum and brain. The results of the analysis showed a significant increase in the phosphatases activity both in the serum (Figure 5A) and brain (Figure 5B) of rats treated with scopolamine alone. In contrast, significant restoration of phosphatase activity was observed in the serum sample of rats treated with scopolamine in combination with KG-HE extract ($p<0.001$) or donepezil ($p<0.0001$) (Figure 5A). Further analysis of the data indicated that the restoration of the phosphatase activity was observed in the serum sample of rats treated with scopolamine and KG-HE extract at the concentrations of 25 mg/kg and 50 mg/kg. For the phosphatase activity in the brain sample, only a mild reduction ($p<0.05$) was observed in the brain samples of rats treated with scopolamine in combination with KG-HE extract at 25 mg/kg (Figure 5B), but a significant reduction was observed when donepezil was used in combination to scopolamine (Figure 5B).

3.7. Inhibition of Fibrillogenesis by the Khaya grandifolia Hydro-ethanolic (KG-HE) Extract in Scopolamine-Stressed Rats

On the brain homogenate from treated and untreated rats, a Congo red binding assay was performed, followed by spectrophotometry analysis. The amount of Congo red binding was then calculated using a standard as described [35]. In addition, a negative binding assay sample was used during analysis by spectrophotometer. The results obtained show that scopolamine caused an increase in the fibril count in rats treated with scopolamine alone, while the fibril count was significantly restored to that of the control level in rats treated with scopolamine combined with KG-HE extract ($p<0.05$) or donepezil ($p<0.001$) (Figure 6). Moreover, the inhibition of fibrillogenesis was more effective in rats treated with scopolamine in association with KG-HE extract at a concentration of 25 mg/kg ($p<0.05$) (Figure 6).

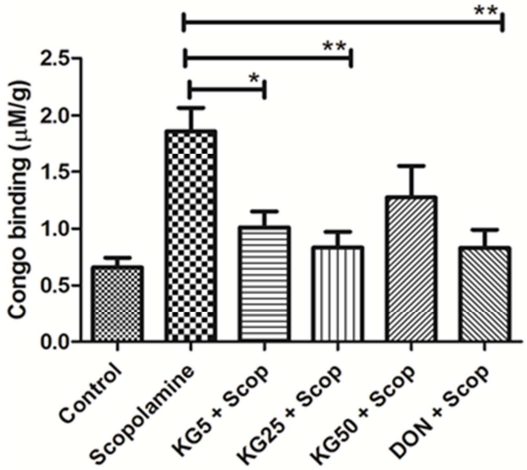

Figure 6. *Evaluation of the anti-fibrillation potential of the Khaya grandifolia hydroethanolic (KG-HE) extracts in rats treated with scopolamine. Rats were administered the solution of scopolamine (1 mg/kg, i.p.) alone or in association with the KG-HE extract (5, 25, and 50 mg/kg, p.o.) or donepezil (1 mg/kg, i.p.). Nine (9) days following treatment, brain homogenate was prepared and used for the evaluation of the Congo red binding. Results are presented as mean ± mean standard error over animals in each group. The significance between rats treated with scopolamine and co-treated with KG extract or donepezil and scopolamine is calculated by the ANOVA test (Tukey's test) *p<0.05; **p<0.001.*

4. Discussion

The current study was undertaken to investigate whether the *Khaya grandifololia* hydro-ethanolic (KG-HE) extract was able to prevent memory impairment, depression, and anxiety in albino strains stressed with scopolamine. Results from behavioral experiments suggested that oral treatment of rats with KG-HE extract actually improved their cognitive functions. The latency time to reach the platform in the KG-treated rat group on day 4 was lower than that of the control group and almost identical to that of the donepezil-treated group. These observations indicate that the KG-HE extract improves learning or acquisition and consolidates the memory of the learned task. In addition, in the tail suspension test that evaluates the antidepressant property of KG-HE extract, KG-treated rats showed a significantly reduced immobility time compared to untreated controls. An increase in immobility time is usually referred to as behavioral despair in animals and is claimed to represent human depression [36-38], suggesting that the KG-treated rats were prevented from depressive-like behaviors. Previous studies by Gardier and colleague [39] showed that a decrease in immobility was linked to antidepressant activity [40-42]. Taken together, these results indicate that the KG-HE extract possesses nootropic potential. Nootropic agents represent a class of psychotic compounds that have a selective effect on the integrative functions of the central nervous system (CNS), particularly on intellectual performance, learning capacity, and memory [43, 44]. Further behavioral tests using scopolamine for the induction of cognitive deficit revealed that KG-HE extract prevented depression, anxiety, and memory deficit. Medicinal plants such as Bacopa monnieri, Ginkgo biloba, Celastrus *paniculatus,* Achyranthes aspera have also been proven to have similar abilities [44-48].

To dissect the mechanism by which KG-HE extract prevents scopolamine-induced cognitive impairment in rats, we evaluated the activity of the cholinergic enzyme marker acetylcholinesterase (AChE). It has been demonstrated that one of the most important mechanisms responsible for the cholinergic function is carried out by the enzyme AChE. The cholinergic system deals with the production of acetylcholine (ACh), and an increase in AChE activity leads to an ACh deficit and cognitive impairment [36]. Therefore, AChE activity plays an important role in the regulation of functions such as memory, learning, and neuronal development and differentiation [49, 50]. Age-related cognitive impairment is due to the dysfunction of cholinergic transmission [51, 52], and the death of cholinergic neurons, which triggers an acetylcholine deficit in the basal forebrain area, is the main cause of Alzheimer's disease (AD) [53, 54]. Experimentally, the deficit in ACh is mimicked by exposing an animal to a certain dose of scopolamine [55], and the current investigation has found that KG-HE extract prevented the scopolamine-induced cognitive impairment through AChE inhibition. Due to the fact that the cholinergic system also modulates inflammation [56], it would be important to investigate whether KG-HE extract, which inhibits AChE, also does the same regarding the inflammatory response activated by scopolamine-induced impairment of the brain functions. From this perspective, KG-HE extract can be used as an anti-inflammatory drug in inflammatory disorders such as Alzheimer's disease and multiple sclerosis.

Memory improvement and prevention of scopolamine-induced cognitive impairment by KG-HE extract observed in this study seem to be modulated by AChE activity, but other mechanisms such as antioxidant defense may also be implicated in these effects. Therefore, we decided to investigate whether KG-HE extract prevents scopolamine-induced cognitive impairment by activating the antioxidant system, since brain functioning requires a substantial amount of oxygen and is more vulnerable to reactive oxygen species (ROS)-mediated damage [57]. In this system, the imbalance between ROS production and the antioxidant defense system in the brain affects essential biomolecules such as enzymes, lipids, proteins, and nucleic acids, leading to the onset and progression of age-related neurodegenerative disorders including Alzheimer's [58, 59]. Scopolamine also induces cognitive dysfunction via the alteration of intracellular antioxidant status in the brain [60]. In this study, treatment of rats with scopolamine (1 mg/kg) induced a decrease in the activity of antioxidant enzymes (CAT and SOD), as well as a decrease in the brain and serum concentration of non-enzymatic antioxidant molecules, including reduced glutathione (GSH). Likewise, MDA levels are also increased in the brain of a rat treated with scopolamine. In contrast, we observed a significant restoration of the activities of CAT and SOD when the rats were treated with scopolamine in association with KG-HE extract. In the same condition, the level of GSH was restored to that of the control, as was the level of MDA, the cause of lipid peroxidation. These findings indicated that KG-HE extract prevented scopolamine-induced cognitive impairment by inhibiting the activation of stress oxidation. Indeed, the nuclear factor erythroid 2-related factor 2 (Nrf2) is activated in liver cells treated with KG extract [21]. Nrf2 is known as an activator of the endogenous defense system that triggers the production of antioxidant enzymes [61].

The main finding in the current investigation is the prevention of memory impairment, depression, and anxiety by KG-HE extract. In the search for the mechanisms implicated in this prevention action initiated by the KG-HE extract, we found that KG-HE extract was also able to regulate the phosphatase activity when rats were stressed with scopolamine. The phosphatase is present on neuronal membranes and the plasma phosphatase activity is increased in brain injury, suggesting that any drop in its activity in the plasma may reflect neuronal loss [62]. In addition, we observed a significant reduction of the congophilic amyloid plaques deposited in the brains of rats treated with scopolamine in association with KG-HE extract and donepezil (positive control) in comparison to the significant density of congophilic amyloid plaques observed in the brains of rats treated with scopolamine alone. This result may indicate that the KG-HE extract exerts its brain protection

mechanism by blocking the amyloid plaque formation initiated by scopolamine. Indeed, using a differentiated neuronal cell line, IMR32, we previously demonstrated in an AD in vitro model that KG crude extract prevents amyloid-induced toxicity by inhibiting tau hyper-phosphorylation [24]. Amyloid plaques and tau hyperphosphorylation are two neuropathological symptoms of AD, and the accumulation of amyloid plaques in the brain triggers a pathological cascade [63]. The neurotoxic effects of amyloid plaques are widely studied [64], and they include impairing plasticity, apoptosis, tau phosphorylation, and stress oxidation [65]. Indeed, KG crude extract has shown in vitro the prevention of some neurotoxic effects that include impairment of synaptic plasticity, apoptosis, tau hyper-phosphorylation, and oxidative stress [24]. Moreover, among the secondary metabolites detected in the KG-HE extract, flavonoids may be responsible for the prevention of the scopolamine-induced neurotoxic effects observed in animals. Indeed, previously published data indicates that age-related brain performance is easily improved by flavonoid compounds, which possess the ability to neutralize free radicals [66]. In addition, it has been reported that cognitive performance can be significantly improved by taking a diet rich in antioxidant species such as flavonoids [67, 68].

5. Conclusion

In summary, this study showed that treatment of rats with an extract of KG-HE prevented the scopolamine-induced cognitive impairments evaluated using the tail suspension test, Morris water maze, and novelty-suppressed feeding test. Furthermore, KG-HE extract exerted its prevention effect by blocking the cholinergic system through the inhibition of AchE activity. Moreover, KG-HE exerted its protective activity when rats were stressed with scopolamine by modulating the antioxidant parameters such as SOD and CAT activity and reducing GSH content. We also demonstrated that KG-HE was also able to prevent scopolamine-induced cognitive impairment in rats by inhibiting the enzyme phosphatase and blocking fibrillogenesis. Further investigations will seek to isolate the secondary metabolites present in the KG-HE extract and test their ability to improve cognitive functions or prevent neurodegeneration.

Conflict of Interest

The authors have declared no conflicts of interest.

Author Contributions

FAE, FNN, SNF, and PFM defined the research subject and the aims, designed the experiments. FAE, MRDK, PO performed the experiments. FNN and SNF analyzed the data and SNF and FAE wrote the paper. All the authors read and approved the final version of the manuscript.

Abbreviations

AchE: Acetylcholine esterase; AD: Alzheimer's disease; ATCI: Acetylthiocholine iodide; BSA: Bovine serum albumin; CAT: Catalase; DON: Donepezil; DTNB: 5,5-dithiobis (2-nitro-benzoicacid); FDA: Food and drug administration; GSH: Reduced glutathione; KG-HE: *Khaya grandifolia hydroethanolic*; MDA: Malondialdehyde; MWM: Morris water maze; NMDA: N-methyl-D-aspartate; NSFT: Novelty-suppressed feeding test; pNPP: para nitrophenyl phosphate; SOD: Superoxye dismutase; TBA: Thiobabituric acid; TCA: Trichloroacetic acid; TST: Tail suspension test.

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