Research Article

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Mice With Scopolamine-Induced Memory Impairment: Carissa edulis (Forssk.)'s Neuroprotective Properties Valh Water Extract (Apocynaceae)

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INTRODUCTION

Depending on the afflicted regions, neurodegenerative illnesses can impact cognition, memory, perception, movement, language, and other aspects of an individual's development by causing nerve cells to deteriorate [1]. Globally, dementia is a significant public health issue, and its prevalence is rising among the elderly [2]. Alzheimer's disease (AD) is one type of dementia that is typified by elevated levels of amyloid beta (Aβ) and phosphorylated Tau protein, which are linked to changes in the central cholinergic system and an irreversible loss of cognitive function that worsens memory [3, 4]. There is currently no treatment for the illness or even a way to stop its advancement. But the majority of the treatment approaches already in use are symptomatic, halting the progression of the illness [5]. Acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists are the primary therapy for the illness [3].

Unfortunately, because of their nonselective activity on a range of organ tissues both centrally and peripherally, these medicines have been linked to a number of side effects, including nausea, vomiting, anorexia, and sleeplessness [6].

Neurotransmitter acetylcholine is extensively distributed in the central nervous system and plays a role in a number of brain functions, including cortical growth and activity, cerebral blood flow regulation, sleep-wake cycles, and cognitive and memory processes [7]. The brain's primary neurotransmission routes involved in memory and cognitive processes include the cholinergic system [8]. In AD, the first pathologic component found was a substantial decrease in cholinergic activity [7]. A change in acetylcholine production or its presynaptic recapture occurs along with neurodegeneration of the cholinergic neurons, leading to a progressive decline in memory function [3, 7]. Due to the material provided, a cholinergic hypothesis of the illness has been established as a study platform in establishing long-term therapies for cholinergic deficiencies in order to improve memory function in AD patients. A muscarinic receptor antagonist called scopolamine inhibits cholinergic neurotransmission, impairing rodent memory. According to recent research, scopolatin causes more reactive oxygen species to build up, which causes oxidative stress and memory impairment [9].

The cholinergic theory can be implemented through the injection of Scopolamine, which causes cognitive abnormalities akin to those seen in AD. The goal of treatment is to restore the cholinergic system's activity by blocking the acetylcholinesterase enzyme.

One potential source of AD medication in the future is medicinal plants. Carissa edulis, often known as C. edulis, was generally chosen for the treatment of dementia due to its traditional medical use.

The plant C. edulis is widely dispersed throughout Africa and is used to treat a variety of conditions including oxidative stress, fever, headaches, malaria, and inflammatory illnesses including rheumatism. Numerous pharmacological studies have examined C. edulis, including in vitro antioxidant activity through scavenging of DPPH and ABTS radicals [10], anticonvulsant activity via various mechanisms, including voltage-gated sodium, potassium, and calcium or GABAergic pathway [11], diuretic activity through increased kidney blood flow and glomerular filtration rate, which leads to increased urine output [12], and more. antiplasmodial activity against chloroquine-sensitive strains of the Plasmodium falciparum parasite [14], antiviral activity against the Herpes simplex virus (HSV) in vitro and in vivo studies [13], and hepatoprotective effect against subchronic administration of dimethoate on guinea pigs by normalizing and restoring the liver enzyme and the antioxidant markers [15]. Even at high dose levels of 5000 mg/kg, C. edulis extracts have all been reported to be well tolerated in experimental animals [16–18]. The current study aims to evaluate the neuroprotective and memory enhancement effects of C. edulis on Scopolamine-induced memory impairment and oxidative stress in mice, with the

goal of searching for safe and novel drugs against memory impairment associated to Alzheimer's disease.

MATERIAL AND METHOD

Plant-Based Materials. The leaves of C. edulis were collected in Cameroon's Far North, and the identity was verified at the National Herbarium Yaounde, where the voucher specimen was stored with the reference 2965/SRFK. To make a fine powder, the leaves were cleaned, sun-dried, crushed, and sieved. Making an Aqueous Extract. After adding 10 grams of the powdered plant material and 60 milliliters of distilled water to a beaker, the mixture was allowed to boil for 20 minutes. Wattman paper no. 1 was used to filter the mixture once it had cooled. The resulting filtrate (C = 62:8 mg/ml) was given to the mice at a volume of 10 ml/kg after being diluted with distilled water at 1/10, 1/4, and 1/2. The C. edulis aqueous extract that was previously prepared as described above (35 ml) of the filtrate was evaporated in an oven at 80°C for 24 hours in order to obtain 2.2 g of dry extract, which was then used to calculate the amount of dry matter in the aqueous extract. The various doses per 10 ml/kg that were created in distilled water were 62.8, 157, 314, and 628 mg/kg.

Making an Aqueous Extract. After adding 10 grams of the powdered plant material and 60 milliliters of distilled water to a beaker, the mixture was allowed to boil for 20 minutes. Wattman paper no. 1 was used to filter the mixture once it had cooled. The resulting filtrate (C = 62:8 mg/ml) was given to the mice at a volume of 10 ml/kg after being diluted with distilled water at 1/10, 1/4, and 1/2. The C. edulis aqueous extract that was previously prepared as described above (35 ml) of the filtrate was evaporated in an oven at 80°C in order to determine the amount of dry matter in the aqueous extract. throughout the course of a day, yielding 2.2 g of dry extract. The various doses per 10 ml/kg of boAnimals that were produced in distilled water were 62.8, 157, 314, and 628 mg/kg. 35 Mus musculus Swiss mice, weighing between 25 and 30 g at two months old, were taken from the Institute of Medical Research and Medicinal Plants Studies' animal house in Yaounde, Cameroon. All of these creatures were housed in cages made of plexiglass, with a constant temperature of roughly 25°C and a light-dark cycle of 12 hours each. The animals were gradually denied food two days before to the studies in order to keep them between 80 and 85 percent of their body weight. Every experiment was conducted in compliance with the internationally accepted principles for laboratory animal use and care.dy weight Chemicals and Drugs. SigmaAldrich provided the following products: hydrogen peroxide, trichloroacetic acid, acetylthiocholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), scopamine hydrobromide, donepezil, and thiobarbituric acid.

Design Experiments. The idea is to administer scopolamine (1 mg/kg), a selective muscarinic acetylcholine receptor antagonist, intraperitoneally (i.p.) to mice in order to cause memory impairment, and then assess the behavioral and biochemical consequences on the mice. Seven groups of five mice each were created from the animals: distilled water was given to the control group; Scopolamine (1 mg/kg i.p.) was given to the Scopolamine group (Scopo); four test groups received C. edulis aqueous extract at varying doses (62.8, 157, 314, and 628 mg/kg) before receiving an injection of Scopolamine (1 mg/kg i.p.); and a positive control group received Donepezil (5 mg/kg) followed by the injection of Scopolamine (1 mg/kg i.p.). Donepezil and C. edulis aqueous extract were administrated orally 30 minutes before Scopolamine injection. All the treatments were daily administered for 7 consecutive days.

Behavioural Studies

T-Maze Assessment. The animals spent five minutes getting acquainted with the equipment in the T-maze during the habituation phase. The animal's preferred arm was the first one it selected, and its discriminated arm was the second.

24 hours later, during the acquisition phase, the food was placed in the preferred arm and the animal's discriminating arm was closed. After being positioned in the starting arm, the mouse was permitted to travel to the open arm. Each mouse participated in this exercise for five minutes. Both the Scopolamine group and the control group received distilled water treatment during the retention phase. the four test groups by the various dosages of the aqueous extract (62.8, 157, 314, and 628 mg/kg), and the positive control group by the dose of donepezil (5 mg/kg). All groups received intraperitoneal injections of Scopolamine (1 mg/kg), with the exception of the control group, which was given distilled water, thirty(30) minutes following the various treatments. The mice were placed into the T-maze one at a time for five minutes, thirty minutes after the Scopolamine was administered. To remove as much of the preceding mouse's stench as feasible, alcohol (70% ethanol) was used to wipe the apparatus after each transit. The time spent in each of the T-maze's arms (preferred and other), the latency time to select the preferred arm.

Movement of Locomotives in an Open Field. The purpose of the locomotor activity in an open area was to confirm the movement of the animals assessed in the Tmaze test with induction of memory impairment. The open field provides for the assessment of an animal's degree of exploration, locomotor activity, and stress levels by representing a novel and unfamiliar habitat and animal emotional reaction [19], all on the same day. Following their administration of Scopolamine and memory analysis in the T-maze, the animals were promptly moved into the open field.

For a duration of five minutes, the subsequent variables were

recorded for every mouse: the amount of "rearing" (when the animal is put on its hind legs by resting its front legs on the device's wall), the number of "crossing" (number of crossed lines or crossed tiles), and the amount of time spent in the center [19].

A task for recognizing objects in an open field. The approach outlined by Ennaceur and Delacour [20] was used to administer the object recognition test. This test consists of three phases and was carried out in an open field box of 50 × 50 × 40 cm. During the habituation phase, the mice were given five minutes to explore the open field at the end of the treatment. Two identical objects (red cubes, 4 x 4 x 4 cm) were positioned in each of the open field's corners at a distance of 10 cm from the sidewall during the acquisition phase (T1). The mice were given five minutes to investigate these two similar objects after being put in the center of the open field. Following that, their cages were refilled with them. A day following T1, the test "choice" (T2) was then made. Mice were reexposed to the two objects, the new (N) and the familiar (F), during T2, which saw the introduction of a new object (blue cone). A stopwatch was used to manually monitor the amount of time the mice spent exploring each object during T1 and T2. The following formula was used to determine a discrimination index (DI):

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Making Tissue Ready. The mice were sacrificed by cervical decapitation right after the behavioral tests, and the brains were promptly taken out and put in boxes with freezing saline to solidify for ten minutes. To create the 10% homogenate, the brain was placed within a graduated cylinder and PBS (pH 7.4) was added. After centrifuging each tube for 15 minutes at 10,000 rpm/min at 4°C, the supernatant was removed for biochemical examinations.

Biochemical Analysis

Preparing the Tissue. Immediately following the behavioral tests, the mice were sacrificed by cervical decapitation, and their brains were removed and allowed to harden for 10 minutes in boxes containing freezing saline. The brain was put inside a graduated cylinder and PBS (pH 7.4) was added to make the 10% homogenate. Each tube was centrifuged for fifteen minutes at 10,000 rpm/min at 4°C, and the supernatant was taken out for biochemical analyses.

A Catalase Activity Estimate. The Aebi technique was used to measure the catalase activity [23]. 1.9 ml of 50 mM phosphate buffer (pH 7.0) was added to 0.1 ml of supernatant. The addition of 1.0 ml of freshly made 30 mM H2O2 started the reaction. Using spectrophotometry, the rate of H2O2 breakdown was determined from variations in absorbance at 240 nm. The expression of catalase activity was units/mg protein.

Calculating the Reduction of Glutathione (GSH). Ellman's reagent was used to measure the reduced glutathione (GSH) in the brain supernatant, as per Ellman's instructions [24]. Ellman's reagent (3 ml) was combined with twenty (20) µl of brain homogenates at room temperature. Using a spectrophotometer, the absorbance of the yellow chemical was measured at 412 nm after an hour. The extinction coefficient value of 13.600 mol-1 cm-1 was used in the Beer-Lambert formula to determine the quantity of glutathione. Acetylcholinesterase Activity Estimation. The Ellman [25] method was used to estimate the quantity of acetylcholinesterase. Twenty microliters of 0.1 M Tris-HCl buffer (pH 8.0), three milliliters of Ellman's reagent (DTNB) in each test tube (blank and assay tubes), and one hundred microliters of brain homogenate are added in order to estimate the acetylcholinesterase activity. The absorbance was measured at 412 nm following 30 and 90 seconds of fast homogenization at room temperature in comparison to the blank.

Concentration of Total Protein. The Human Diagnostics Worldwide Total Protein Kit methodology was used in order to quantify total proteins in mouse brain homogenates.The statistical analysis. GraphPad Prism version 5.0 was used to analyze the data. The data (n = 5) was displayed as the mean ± SEM. When dealing with normally distributed data, a oneway ANOVA was used to compare the various groups under study, and the Tukey multiple comparison test was then employed.

Analysis was done by comparing the groups receiving the extract to the Scopolamine group and the control group to the Scopolamine group. When the difference was considered statistically significant, it was at $p < 0.05$.

RESULTS

Impact of C. edulis on T-Maze Test-Induced Scopolamine-Induced Memory Impairment Time of Latency. Figure 1 illustrates the impact of the Carissa edulis aqueous extract on the latency time for the mice to access the preferred arm in the T-maze. The findings indicate that the Scopolamine group's latency time of entry into the preferred arm was significantly longer ($p < 0:001$) than that of the control group. When the animals are given different dosages of C. edulis and Donepezil compared to the Scopolamine group, the latency time considerably decreases ($p < 0:001$), going from 23:6±1:14 sec to 12:8±1:30 sec for the mice given a dose of 628 mg/kg of C.

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The amount of time spent in each arm of the T-Maze

The effects of the C. edulis aqueous extract on the amount of time an animal spends in each arm of the T-maze, as well as how the animal discriminates and prefers certain arms during the retention phase, are depicted in Figure 2. In the group of mice given the doses of 62.8 mg/kg, 157 mg/kg, 314 mg/kg, and 628 mg/kg C. edulis aqueous extract and 5 mg/kg Donepezil, respectively, the amount of time spent in the preferred arm increases significantly ($p < 0:01$, $p < 0:001$) in the following order: 75:6±4:4 sec, 80:2±3:2 sec, 96:2±1:7 sec, 106:8±8:5 sec, and 117:4±9:1 sec. This is in contrast to the Scopolamine group, which only passed 59:6±3:20 sec. Thus, for the preferred arm, Scopolamine considerably ($p < 0:001$) shortened the time when compared to the control group. When comparing the amount of time spent in the categorized arm, the reverse impact is seen, with Scopolamine considerably increasing the amount of time.

The total number of entries in each arm of the T-Maze

The number of alternate entry into the T-maze's arms is displayed in Figure 3. Comparing the Scopolamine group to the control, there is a significant rise ($p < 0:05$) in entries into the discriminated arm and a significant drop ($p < 0:01$) in the number of entries into the preferred arm. When compared to the Scopolamine group, treatment with 628 mg/kg of C. edulis aqueous extract and donepezil increased the number of entry ($p < 0:05$) into the preferred arm. Within the categorized arm, a notable A reduction ($p < 0:05$, $p < 0:01$) in the quantity of entries was noted after administering the doses of 157 mg/kg, In comparison to the Scopolamine group, the C. edulis aqueous extract contained 314 mg/kg and 628 mg/kg.

Movement of Locomotives in an Open Field. The mice were assessed for their locomotor activity in the open field following the T-maze test since they had been given scopolamine.

When compared to the Scopolamine group, Table 1 demonstrates a substantial increase ($p < 0:001$) in locomotor activity based on the number of crossings in the mice receiving dosages of C. edulis aqueous extract and getting donepezil. When compared to the control group, the crossing number of the Scopolamine group (50:8±3:1) considerably decreased ($p < 0:001$). The same is true for rearing, where animals given a dose of 628 mg/kg C. edulis aqueous extract have increased activity ($p < 0:05$), while the Scopolamine group ($p < 0:001$) experiences a decrease in activity. In animals administered with Scopolamine, the amount of time spent in the middle of the open field dramatically decreased ($p < 0:001$). When the animals were given 628 mg/kg of C. edulis aqueous extract ($p < 0:01$) and Donepezil ($p < 0:001$) in comparison to the Scopolamine group, a longer period of time was seen.

DISCUSSION

The goal of the current investigation is to determine whether or not Carissa edulis can enhance memory impairment by acting on the cholinergic pathways. A notable role for medicinal plants is being played in the treatment of Alzheimer's disease and memory impairment. In this investigation, we assessed the impact of C. edulis on the Tmaze and new object recognition tests' effects on amnesic mice's memory function. As previously documented [26], scopolanine blocked the muscarinic cholinergic receptors in the brain, causing amnesia in the animals through decreased memory. In the current study, mice given continuous doses of scopolantine had longer latencies to enter the preferred arm, which decreased the amount of time spent in the preferred arm. Time spent in the preferred arm of the T-maze increases when C. edulis aqueous extract is administered because it reduces this latency time. As previously stated [27], the decrease in latency time suggested an improvement in memory, and consequently the memory abilities [29]. A healthy memory is indicated by the notable rise in both the quantity of entries and the amount of time spent in the favorite arm [28, 29].

The findings indicate that the aqueous extract of C. edulis has antagonistic effects on the action of Scopolamine. This may be because the extract contains bioactive substances like coumarins, polyphenols, terpenes, tannins, flavonoids, cardiac glycosides, lignans, and sesquiterpenes that may improve memory loss by blocking the effects of Scopolamine. Numerous studies show that polyphenols have the antioxidant ability to permeate the blood-brain barrier and neutralize free radicals, protecting the brain and nervous system. Memory enhancement is one of polyphenols' primary roles [9]. Furthermore, the rise in entrances and the amount of time spent in the arms, preferred and differentiated, indicating a rise in the exploring behavior.

Mice's locomotor activity and exploratory behavior in an unfamiliar environment are assessed using the open field test [30]. The mice given scopolamine (1 mg/kg) exhibit decreased crossing and rearing, which changes the locomotor activity of the drug. In this test, the group of mice administered with the C. edulis aqueous extract showed increased exploration and locomotor activity as evidenced by an increase in the number of "crossing," "rearing," and time spent in the center. The aqueous extract of C. edulis may have memory-related qualities, which may be mediated by cholinergic neurotransmission at the hippocampal and cerebral cortical levels [30].

Memory functions heavily rely on the central cholinergic system [8]. Age-related cognitive deficits are caused by dysfunctional acetylcholinergic neurons [4, 6]. The available data align with the findings of Chen et al. [42], Budzynska et al. [43], and Park et al. [44], who documented that scopolanine causes significant cholinergic deficits and increases acetylcholinesterase

activity in the hippocampus, hence exacerbating brain neurodegeneration. When acetylcholinesterase was treated with the aqueous extract of C. edulis, it was much less active than when the Scopolamine group was treated.

This finding is in line with a previous study in which the hydroethanolic extract improved memory in various models of cognitive impairment and reduced AChE activity [40]. Therefore, our findings imply that the inhibition of acetylcholinesterase activity and the enhanced release of acetylcholine into the synaptic gap, along with its fixation on the postsynaptic receptors, account for the memory-enhancing effects of C. edulis.

CONCLUSION

The goal of this study was to determine whether the aqueous extract of Carissa edulis, a muscarinic acetylcholine receptor antagonist, might prevent memory loss caused by the drug scopamine. Deficits in memory and learning caused by scopolanine assessed in T-maze behavioral studies additionally, Both oxidative stress and acetylcholinesterase activity increased in response to item recognition tests. Administering C. edulis aqueous extract improved memory substantially, as evidenced by T-maze and novel object recognition tests. It also strengthened the antioxidant defense system, shielding neurons from oxidative stress and mitigating the memory loss caused by Scopolamine. This investigation was restricted on C's protective qualities. edulis on the cholinergic pathway by mitigating the effects of Scopolamine on cognitive impairment. Therefore, it is still unknown how C. edulis affects other potential pathways, such as glutamatergic and inflammatory ones, that could be involved in the pathophysiology of AD.

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