Anti-Amnesic and Neuroprotective Effects of Ziziphus Jujuba Aqueous Extract on Scopolamine-Induced Cognitive Impairments in Rats

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Abstract

Background: Alzheimer's disease is a neurological condition that affects more than 44 million people worldwide. The available treatments target the symptoms rather than underlying causes. *Ziziphus jujuba* (Rhamnaceae) is used in traditional Cameroonian medicine to treat many disorders including memory impairments. The study aimed to evaluate the anti-amnesic and neuroprotective effects of *Z. jujuba* aqueous extract on scopolamine-induced memory disorders in rats.

Methods: Learning and memory impairments were induced in rats by scopolamine (1mg/kg, i.p.) for 15 days. Rats that developed cognitive impairments were divided as follows: two positive control groups received piracetam (200 mg/kg, *p.o.*) or tacrine (1 mg/kg, *p.o.*); three test groups received the extract (29, 57, and 114 mg/kg, *p.o.,* respectively) daily for 15 days. At the end of treatments, memory impairments were assessed by Morris water maze and Y-maze tests. Thereafter, animals were sacrificed and some biochemical parameters (oxidative stress, inflammation, and apoptosis) were estimated in the hippocampus and prefrontal cortex.

Results: *Z. jujuba* decreased the time to reach the platform and increased the time in the target quadrant. However, it failed to affect spontaneous alternation in the Y-maze. Furthermore, the extract reversed scopolamine-induced oxidative stress, inflammation, and apoptosis. This was confirmed with the prevention of neuronal loss in the hippocampus or prefrontal cortex.

Conclusions: These findings suggest that *Z. jujuba* extract possesses ant-amnesic and neuroprotective effects. It seems that these effects are mediated in part by antioxidant, anti-inflammatory, and anti-apoptotic activities. This, therefore, justify its use to treat dementia and psychiatric disorders in Cameroon’s folk medicine.

Introduction

Alzheimer's disease (AD) is the most common form of dementia (60 -70% of cases) [1]. AD is an irreversible and progressive neurodegenerative disorder of the central nervous system, which occurs gradually and leads to memory loss, unusual behavior, and personality changes [1, 2]. According to the World Health Organization (WHO), more than 44 million people worldwide are affected by AD with 7.7 million new cases every year [3-5]. In Africa, the prevalence of AD is estimated at 5.6% [6]. At the molecular and cellular levels, AD is characterized by extracellular deposits of beta-4-Amyloid (PβA) protein, intracellular entanglements, cholinergic deficit, extensive neuronal loss, and synaptic changes in the cerebral cortex and hippocampus [7]. PβA deposit causes neuronal death via some possible mechanisms including, oxidative stress, inflammation, and apoptosis [2]. At present, there is no curative treatment against AD [8]. Drug therapies are suggested such as acetylcholinesterase inhibitors (Galantamine, Rivastigmine, and Donepezil) and N-methyl-D-aspartic acid (Memantine) receptor antagonists [9]. These treatments are very expensive, difficult to access, have side effects, and are all symptomatic relieves [2, 3]. Therefore, medicinal constitute a source for the discovering of effective drugs

against AD. Among them, *Ziziphus jujuba*, a plant from the Rhamnaceae family, is used in Northern Cameroon, Asia, and India for the treatment of many pathologies including typhoid fever, furuncle, sleep disorders, diarrhea, and pain [10, 11]. In Cameroon, all parts of the plant are used to treat otitis, inflammation, cancer, anxiety, rickets, typhoid fever, and anorexia. Seeds are used as dewormers [12] and leaves in cases of dementia [12]. In recent years, research on *Z. jujuba* fruits have shown to possesses anti-inflammatory [13] and neuroprotective activity [14], while the leaves showed to possess anti-inflammatory [15], antifungal, anticancer, antifertility, antibacterial, anxiolytic, sedative, and antioxidant properties [16-18]. Research undertaken on the anti-amnesic effect of *Z. jujuba* revealed that the seed possesses a protective effect against spatial memory impairments in rats [19-21]. According to other authors, this effect could be mediated by cholinergic blockade [22]. Moreover, the hydroethanolic extract of *Z. jujuba* was demonstrated to ameliorate cognitive decline and seizures in an experimental model of epilepsy in rats [20]. A toxicity study on *Z. jujuba* leaves revealed that they are non-toxic [23]. Nonetheless, toxic elements are found in trace amounts in the whole plant [24]. Phytochemical analysis from seeds, leaves, and stem barks revealed the presence of alkaloids, flavonoids, tannins, saponins, and polyphenols [25]. The HPLC fingerprint file of *Z. jujuba* leaves extract identified the presence of major constituents such as (−)-catechin, traumatic acid, quercetin-3-*O*-robinobioside, rutin, and quercetin-3-*O*-*α*-*L*-arabinosyl-(1→2)-*α*-*L*-rhamnoside with the total contents of nine flavonoids [26]. Furthermore, GC/MS analysis of ethanol extract of *Z. jujuba* seeds revealed the existence of 20 component, main components were 13-Heptadecyn-1-ol (12.95%), 7-Ethyl-4-decen-6- one (9.73%), Lineoleoyl chloride (8.54%), Linoleic acid (6.37%), 2,5-Octadecadiynoic acid, methyl ester (5.57%) and Palatinol A (4.81%)[27]. To date, there is no scientific evidence on the anti-amnesic and neuroprotective effects of *Z. jujuba* leaves aqueous extract. Therefore, this study was undertaken to investigate the anti-amnesic and neuroprotective effects of *Z. jujuba* leaves aqueous extract on scopolamine-induced cognitive impairments in rats, using Morris water maze and Y-maze paradigms. Possible mechanisms of action have been also explored.

1. Material And Methods

1.1. Plant collection and extraction

The leaves of *Z. jujuba* were harvested in Mokolo, in the Far North region of Cameroon, at global position system coordinates 10.7425° North, 13.8042° East. It was identified at the National Herbarium of Cameroon (HNC) (database of herbarium index: http://sweetgum.nybg.org/science/ih/herbarium-list) by Mr. Ngansop Eric in comparison to sample N°14446/HNC. The plant name has been checked on http://www.theplantlist.org. The extract of *Z. jujuba* has been prepared according to the traditional healer's method. Briefly, fresh leaves of *Z. jujuba* were dried in shade and crushed into powder. The *Z. jujuba* leaf powder (75 g) was boiled in 1.5 l of distilled water for 20 min. After cooling, the obtained solution was filtered with Whatman No. 3 paper. The filtrate (1.17 l) was dried at 50°C in an oven to a dried extract (10.8 g), yield 14.40%. From the stock solution (5.7 mg/ml), less and most concentrated solutions were respectively made at ratios 1/2, and 2. These solutions were therefore administered in rats at doses 29 and 114 mg/kg, respectively.

1.2. Drugs and chemicals

Piracetam tablets were purchased from U.C.B. Pharma SA Braine-l'Alleud (Belgium) and tacrine hydrochloride capsules from Shionogi. Inc (Japan). Scopolamine, trichloroacetic acid, thiobarbituric acid, Ellman reagent, adrenaline, and formalin were purchased from Sigma Chemical Co., St. Louis (United States), while ethyl ether from Cooper Laboratory (Paris). Piracetam, tacrine, and scopolamine were dissolved in distilled water. All solutions were administered *per os* (*p.o.*) at a volume of 10 ml/kg except, scopolamine administered intraperitoneally (i.p.).

1.3. Animals

Animals were male Wistar rats, 6 to 8 weeks old, weighing between 120 - 140 g. These animals were raised in the animal house of the laboratory of Animal Physiology (University of Yaoundé I, Cameroon) under standard light (12-hour day/night cycle) and temperature (24-26°C) with free access to standard animal diet and tap water. Animals procedures were carried out following the guidelines of the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Technological Innovation (Reg. no. FWA-IRD 0001954, 04/09/2006), which adopted the guidelines of the European Union on Animal Care (C.E.E. Council 86/609). Anesthesia of animals has been made according to the American Veterinary Medical Association (AVMA) guidelines for the euthanasia of animals (2020). The ethical procedure (following an institutional guidelines), the study design (group being compared including control group), the sample size (number of animals per group), the experimental procedure (animal model well known and described), the experimental animal (sex, strain and age of animals detailed), housing (Animal facilities of the University of Yaounde I in recommended conditions), allocating animals to experimental group, experimental outcomes (dosage of biomarkers), and statistical methods in this study are detailed in the manuscript and carried out according to the ARRIVE guidelines (http://www.nc3rs.org.uk/page.asp?id=1357)

1.4. Experimental design

To induce cognitive impairments, rats were divided into 2 groups and treated for 15 days:

- group of 50 rats treated with scopolamine (1mg/kg, i.p.);

- normal control group of 8 rats received distilled water (10 ml/kg, i.p.).

At the end of treatments, learning and memory impairments were assessed on Morris water maze and Y-maze paradigms. Rats that developed learning and memory impairments were selected for the rest of the experiment. These rats were then divided into 5 groups of 8 rats as follows:

- two positive control groups received piracetam (200 mg/kg, *p.o.*) or tacrine (10 mg/kg, *p.o.*);

- three test groups received the aqueous extract of Z. *jujuba* (29, 57, and 114 mg/kg, respectively);

Furthermore, the aforementioned normal control group was added as the 6th group and treated with distilled water (10 ml/kg, *p.o.*).

All animals were thus treated for 15 additional days. At the end of the experimental period, cognitive declines were again evaluated on the aforementioned paradigms. After completion of behavioral analysis, rats were sacrificed, and the brain was removed for biochemical markers and histological assays (Figure 1).

1.5. Behavioral studies

1.5.1. Morris water maze test

Spatial long-term learning and memory were studied using the Morris Water Maze [28]. A circular tank (150 cm diameter and 60 cm height) was filled to 40 cm with water (25°C). The pool was virtually divided into four equal quadrants: North, South, East, and West. A white refuge platform (8 cm diameter and 30 cm height) was placed in the center of one of the quadrants, i.e.1 cm below the surface of water. The water was bleached by adding liquid milk so that the platform was invisible on the surface of water. The pool was located in a room with various visual cues. On the 1st day of the test (habituation phase), each rat has been acclimatized for 60 s in the absence of the platform. The following tests (acquisition phase) took place in 4 days with a daily session of 3 sessions per day. The session time for each animal to find the platform was 120 s. When an animal found the platform, it was left to stay on its top for 10 s. If after 120 seconds an animal was unable to locate the platform, it was taken there and allowed to remain for 10 s. The time interval between sessions was 5 minutes. During each session of the acquisition phase, the latency time to find the platform was recorded for each animal. The effectiveness of learning was assessed in the retention phase. During this phase, which lasts 120 s, the platform was removed from the tank. The latency time to find the platform and the time spent in this compartment were recorded.

1.5.2. Y-maze test

The Y-maze test was used to assess working memory in animals by recording spontaneous alternation [29]. The maze used was a wooden device with 3 identical branches (40 cm long x 35 cm high x 12 cm wide) separated by an angle of 120°. The walls of each arm were decorated with a different pattern (A, B, and C) to differentiate them. Rats were individually placed at the end of a branch of the maze, to freely explore the maze for 5 min [30]. The number of entries in each arm of the maze was recorded. After each animal session, the device was cleaned with 10% ethanol. A spontaneous alternation (SA) has been defined, as three successive entries in the three different arms (example: ABC, CAB, or BCA). The percentage of SA was used as an index of performance of the working memory and calculated according to the following formula: [(Number of AS)/(total number of arms visited -2)] 100.

1.6. Biochemical analysis

1.6.1. Sacrifice and preparation of homogenates

At the end of the behavioral assessment, animals were sacrificed by decapitation after ethyl ether anesthesia. The brain of each animal was removed and divided into two hemispheres. Hippocampus and prefrontal cortex from the first half were isolated, washed in 0.9% NaCl, and wrung out. They were then weighted and homogenized with Tris-HCl buffer (50 mM, pH 7.4) in a ratio of 20%. Following

centrifugation at 3000 rpm at 4°C for 25 min, the supernatant was removed and stored at -20°C for neurochemical parameters evaluation. The second half of the brains were fixed in 4% formalin for histological analysis.

1.6.2. Reduced tissue glutathione level

The Ellman reagent (1.5 ml) was introduced into tubes containing 100 µl of homogenate (test tubes) or Tris buffer (50 mM HCl, 150 mM KCl, pH 7.4) (blank tube). These tubes were shaken and incubated for 60 min at room temperature. Absorbance was read against the blank at 412 nm. The level of reduced glutathione (GSH) was expressed in mol/g of tissue protein [31].

1.6.3. Malondialdehyde level

The MDA assay was carried out according to the method described by Wilbur et al. [32]. Briefly, 250 µl of homogenate was introduced into test tubes and 250 µl of tris buffer (50 mM HCl; 150 mM KCl; pH 7.4) into a blank tube. To each tube was added 125 µl of 20% trichloroacetic acid and 250 µl of 0.67% thiobarbituric acid. Tubes were incubated for 10 min at 90°C. They were then cooled and centrifuged at 3000 rpm for 15 min at room temperature. The supernatant was removed and absorbance was read at 530 nm against the blank. The level of MDA was expressed in mmol/g of tissue proteins.

1.6.4. Superoxide dismutase activity

The activity of SOD was determined according to Misra and Fridovish method [33]. Into blank tube, was introduced 1666 µl of carbonate buffer (50 mM, pH 10.2), and 134 µl of homogenate in test tubes. The reaction was started by adding 200 µl of 0.3 mM adrenaline solution. After fast inversion for homogenization, optical density was read at 480 nm after 20 and 80 s. The specific activity of SOD was expressed in units of SOD/min/g of organ.

1.6.5. Nitrite level

The determination of nitrite level was carried out according to Grand et al. method [34]. The absorbance was read at 570 nm against the blank. The nitrite level was expressed in mg/ml.

1.6.6. Total protein level

The total protein assay was carried out according to Gornall et al. method [35]. The protein concentration was expressed in mg/ml.

1.6.7. Pro-inflammatory markers level

The level of TNF-α, IL1-β, and IL-6 was determined by Enzyme-Linked Immunosorbent Assay (ELISA) using the Quantikine kit (R and D Systems, Inc. Minneapolis, USA).

1.6.8. Apoptosis markers level

The determination of the level of caspases 3 and 9 was carried out by the ELISA technique using the Novus Biologicals kit (R and D Systems, Inc. Minneapolis, USA).

1.7. Histopathological analysis of brain tissue

The histological analysis included fixing, cutting, dehydration, inclusion, cutting, coloring, mounting, and observation. The stained and mounted slides were observed at 250x magnifications, using Scientico STM-50 optical microscope (HSIDC Industrial Estate, Haryana, India) equipped with a Celestron 44421 digital camera connected to a computer.

1.8. Statistics

Statistical analysis was performed using Graphpad Prism software version 7. 1. Difference between groups was analyzed using one-way or two-way analysis of variance (ANOVA) followed by *post-hoc* test of Turkey. The difference was considered significant at $p < 0.05$.

2. Results

2.1. Phytochemical composition of *Z. jujuba* aqueous extract

Qualitative phytochemical screening of *Z. jujuba* aqueous extract showed the presence of flavonoids, phenols, anthraquinones, coumarins, saponins, tannins, triterpenes, anthocyanins, phenols, and reducing sugars (Table 1).

Table 1: Phytochemical composition of *Z. jujuba* leaves aqueous extract

Secondary metabolite	Result
Flavonoids	+
Phenols	+
Alkaloids	-
Anthraquinones	+
Coumarins	+
Saponins	+
Tannins	+
Triterpenes	+
Anthocyanins	+
Reducing sugars	+

(+) present; (-) absent

2.2. Effect of *Z. jujuba* extract on the acquisition phase in the Morris water maze

On the 4th day of the acquisition phase, scopolamine significantly ($p < 0.001$) induced learning deficit in the negative control group compared to the normal control group (Table 2). the extract of *Z. jujuba* at all doses increased the latency to reach the platform to 5.2 ± 0.4 s ($p < 0.001$) at dose 29 mg/kg compared to the negative control (Table 2). Piracetam increased ($p < 0.01$) this time to 7.7 ± 0.7 s (Table 2).

Table 2: Effect *Z. jujuba* extract on latency time to reach the platform in the Morris water maze during acquisition phase

Treatment	Day 1 Lat. (s)	Day 2 Lat. (s)	Day 3 Lat. (s)	Day 4 Lat. (s)
DW+DW	21.3 ± 4.4	8.3 ± 0.8	6.9 ± 0.5	5.9 ± 0.2
SCO+DW	29.3 ± 3,5	15.0 ± 2.0	14.1 ± 2.2	14.5 ± 2.7[c]
SCO+PI	21.4 ± 3.9	9.2 ± 1.4	10.3 ± 1.3	7.7 ± 0.7**
SCO+TA	51.7 ± 4.3	10.6 ± 1.8	9.8 ± 1.5	10.9 ± 1.4
SCO+E29	38.8 ± 2.1	9.8 ± 0.9	6.2 ± 0.8	5.2 ± 0.4***
SCO+E57	36.9 ± 6.7	6.9 ± 0.7	8.8 ± 0.9	7.2 ± 0.7**
SCO+E114	27.2 ± 3.4	6.7 ± 1.5	7.4 ± 0.6	6.0 ± 0.7***

Each value represents the average ± ESM; n = 7-8. cp <0.001 *vs* normal control. ** p <0.01; *** p <0.001 *vs* negative control. s: Second; Lat.: Latency time; DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine; SCO + E29-E114: Test groups treated *Z. jujuba* extract.

2.3. Effect of *Z. jujuba* extract on the retention phase in the Morris water maze

During the retention phase, administration of scopolamine increased ($p < 0.001$) latency to reach the platform and decreased time spent in the target quadrant in the negative control group compared to the normal control group (Figures1 A and B). The extract of *Z. jujuba* increased latency to reach the platform to 3.9 ± 0.4 s ($p < 0.001$) at dose 114 mg/kg (Figure 1A). It also increased the time spent in the target quadrant to 40.9 ± 2.0 s ($p < 0.001$) at dose 57 mg/kg (Figure 1B). Piracetam increased this time to 38.7± 1.9 s ($p < 0.05$) (Figure1B).

Each value represents the average ± ESM; n = 7-8. cp <0.001 *vs* normal control. * p <0.05; ** p <0.01; *** p <0.001 *vs* negative control. DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI:

Positive control group with piracetam ; SCO + TA: Positive control group with tacrine ; SCO + E29-E114: Test groups treated *Z. jujuba* extract.

2.4. Effect of *Z. jujuba* extract on spontaneous alternations in the Y-maze

Administration of scopolamine in the negative control group reduced ($p < 0.01$) spontaneous alternations compared to the normal control group (Figure 2). In the group treated with the extract as well as piracetam or tacrine, a nonsignificant increase ($p > 0.05$) in spontaneous alternation was observed (Figure 2).

Each value represents the average ± ESM; n = 7-8. bp <0.01 *vs* normal control. DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine ; SCO + E29-E114: Test groups treated *Z. jujuba* extract.

2.5. Effect of Z*. jujub*a extract on the level of GSH, MDA, SOD, nitrite, and total protein in the hippocampus and prefrontal cortex

Administration of scopolamine in the negative control group decreased ($p < 0.001$) the GSH level in the hippocampus and prefrontal cortex compared to the normal control group (Tables 3 and 4). The extract at doses 29 and 114 mg/kg increased the level of GSH in the hippocampus to 77.59% ($p < 0.05$) et de 72.04% ($p < 0.05$), respectively. In the prefrontal cortex, the extract (29 mg/kg) increased ($p < 0.001$) the GSH level to 264.57%. Tacrine increased the GSH level in the hippocampus and prefrontal cortex to 127.62% ($p < 0.05$) and 209.91% ($p < 0.01$), respectively (Tables 3 and 4).
Injection of scopolamine in the negative control group increased ($p < 0.001$) the level of MDA in the hippocampus and the prefrontal cortex compared to the normal control group (Tables 3 and 4). The extract at all doses decreased ($p < 0.001$) the MDA level in the hippocampus. In the prefrontal cortex, it (114 mg/kg) reduced ($p < 0.01$) the MDA level (Tables 3 and 4). Piracetam and Tacrine decreased ($p < 0.001$) the MDA level in the hippocampus. In the prefrontal cortex, piracetam ($p < 0.05$) and tacrine ($p < 0.01$) decreased in this level (Tables 3 and 4).

The activity of superoxide dismutase (SOD) increased ($p < 0.001$) in the hippocampus and prefrontal cortex in negative control compared to normal control (Tables 3 and 4). The extract at all doses reduced ($p < 0.001$) the activity of SOD in the hippocampus and the prefrontal cortex (Tables 3 and 4). Piracetam and Tacrine also reduced ($p < 0.001$) this activity in the hippocampus and prefrontal cortex (Tables 3 and 4).
The level of nitrite increased ($p < 0.001$) in the hippocampus and the prefrontal cortex of the negative control group compared to the normal control group (Tables 3 and 4). The extract at doses 29 and 114 mg/kg decreased ($p < 0.001$) the level of nitrite in the hippocampus and prefrontal cortex (Tables 3 and 4). Piracetam and Tacrine also led to a reduction ($p < 0.001$) in the level of nitrite in the hippocampus and prefrontal cortex (Tables 3 and 4).
Administration of scopolamine in the negative control group decreased ($p < 0.001$) total protein level in

the hippocampus and prefrontal cortex compared to the normal control group (Tables 3 and 4). The extract (29 and 144 mg/kg) increased ($p < 0.001$) total protein level in the hippocampus and prefrontal cortex (Tables 3 and 4). Piracetam and Tacrine increased ($p < 0.001$) total protein level in the hippocampus and prefrontal cortex (Tables 3 and 4).

Table 3: Effect of *Z. jujuba* extract on the level of some oxidative stress markers in the hippocampus

Treatment	Oxidative stress markers in the hippocampus				
	GSH (µmol/g)	MDA (pmol/g)	SOD (Unit/min/g)	Nitrite (mmol/ml)	Total protein (µg/ml)
DW+DW	690.1 ± 48.3	17.1 ± 1.2	14.4 ± 0.8	144,1 ± 1.7	1751.3 ± 24.1
SCO+DW	316.4 ± 18.9^{c}	64.6 ± 3.4^{c}	146.1 ± 84.3^{c}	532,4 ± 3.4^{c}	450.8 ± 13.2^{c}
SCO+PI	370.6 ± 30.8	20.3 ± 2.2***	214.6 ± 17.5***	307,2 ± 2.1***	1367.9 ± 9.7***
SCO+TA	720.1 ± 85.6*	40.5 ± 7.4***	743.6 ± 129.8***	409,2 ± 2.2***	1176.2 ± 34.4***
SCO+E29	561.9 ± 30.3*	16.3 ± 1.3***	191.1 ± 15.5***	332,8 ± 2.2***	1139.9 ± 39.3***
SCO+E57	319.4 ± 11.5	17.9 ± 1.1***	187.9 ± 10.8***	331,2 ± 3.8***	1352.3 ± 26.4***
SCO+114	544.3 ± 33.2*	28.3 ± 1.8***	260.4 ± 13.2***	327,2 ± 2.6***	1466.3 ± 21.1***

Each value represents the average ± ESM; n = 7-8. cp <0.001 *vs* normal control. * $p < 0.05$; *** $p < 0.001$ *vs* negative control. s: Second; Lat.: Latency time; DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine; SCO + E29-E114: Test groups treated *Z. jujuba* extract; GSH: Reduced glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase.

Table 4: Effect of *Z. jujuba* extract on the level of some oxidative stress markers in the prefrontal cortex

Treatment	Oxidative stress markers in the prefrontal cortex				
	GSH (µmol/g)	MDA (pmol/g)	SOD (Unit/min/g)	Nitrite (mmol/ml)	Total protein (µg/ml)
DW+DW	1134.1 ± 102.4	4.5 ± 0.5	61.3 ± 5.1	169.6 ± 1.7	2170.9 ± 36.1
SCO+DW	232.6 ± 33.7^{c}	13.3 ± 2.3^{c}	212.6 ± 373.2^{c}	613.6 ± 2.8^{c}	331.6 ± 32.2^{c}
SCO+PI	556.8 ± 74.5	6.8 ± 1.1*	131.1 ± 13.5***	416.1 ± 3.2***	1129.5 ± 31.3***
SCO+TA	720.9 ± 120.2**	6.2 ± 1.1**	59.4 ± 7.3***	530.4 ± 3.2***	1404.1 ± 25.1***
SCO+E29	848.1 ± 87.9***	9.5 ± 0.7	271.8 ± 24.1***	370.4 ± 3.5***	958.6 ± 28.4***
SCO+E57	710.4 ± 79.9**	9.5 ± 1.2	395.1 ± 48.3***	366.4 ± 2.8***	865.3 ± 26.7***
SCO+114	538.1 ± 56.9	5.3 ± 0.5**	321.5 ± 27.5***	425.2 ± 1.1***	683.9 ± 32.4***

Each value represents the average ± ESM; n = 7-8. cp <0.001 *vs* normal control. * p <0.05; ** p <0.01; *** p <0.001 *vs* negative control. s: Second; Lat.: Latency time; DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine; SCO + E29-E114: Test groups treated *Z. jujuba* extract; GSH: Reduced glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase.

2.6.Effect of *Z. jujuba* extract on the level of pro-inflammatory markers in the hippocampus and prefrontal cortex

The rats of the negative control group showed an increase of TNF-α level in the hippocampus and prefrontal cortex to 158.4 ± 1.3 pg/ml (p <0.001) and 2148.9 ± 1.7 pg/ml (p <0.001), respectively compared to normal rats (Figure 3A). The extract (114 mg/kg) reduced the TNF-α level in the hippocampus and prefrontal cortex to 62.47% (p <0.001) and 78.96% (p <0.001), respectively (Figure 3A). Piracetam and Tacrine increased the TNF-α level in the hippocampus (42.59% and 28.76%, respectively) and prefrontal cortex (73.53% and 73.16%, respectively) (Figure 3A).
The rats of the negative control group showed an increased IL-1b level in the hippocampus (112.1 ± 0.8 pg/ml) and prefrontal cortex (1281.3 ± 1.1 pg/ml) compared to normal rats (Figure 3B). The extract (29 mg/kg) decreased the IL-1b in the hippocampus and prefrontal cortex to 79.69% (Figure 3B). In the

prefrontal cortex, it (114 mg/kg) increased the IL-1b level to 60.12%. Piracetam and Tacrine decreased ($p < 0.001$) the IL-1b level in the hippocampus (52.37% and 61.04%, respectively) and prefrontal cortex (39.16% and 45.42%, respectively) (Figure 3B).
Administration of scopolamine in the negative control group increased the IL-6 level in the hippocampus and prefrontal cortex to 346.1 ± 8.7 pg/ml ($p < 0.001$) and 3526.3 ± 3.4 pg/ml ($p < 0.001$), respectively compared to the negative control (Figure 3C). The extract at all doses decreased ($p < 0.001$) the IL-6 level in the hippocampus and prefrontal cortex (Figure 3C). Piracetam and Tacrine decreased ($p < 0.001$) the IL-6 level in both organs (Figure 3C).

2.7. Effect of *Z. jujuba* extract on the level of apoptotic markers in the hippocampus and prefrontal cortex

Administration of scopolamine increased ($p < 0.001$) the caspase 3 level in the hippocampus and prefrontal cortex to 3.7 ± 0.1 ng/ml and 25, 4 ± 0.1 ng/ml, respectively compared to negative normal control group (Figure 4A). The extract of *Z. jujuba* (144 mg/kg) decreased this level in the hippocampus to 87.94% ($p < 0.001$), while in the prefrontal cortex it (29 mg/kg) decreased this level to 60.95% ($p < 0.001$) (Figure 4A). Piracetam and Tacrine decreased ($p < 0.001$) the level of caspase 3 in the hippocampus (37.19% and 24.03%, respectively) and prefrontal cortex (62, 70%, and 59.05%, respectively) (Figure 4A).
In the negative control group, the caspase 9 level increased in the hippocampus and prefrontal cortex to 22.9 ± 0.1 ng/ml ($p < 0.001$) and 81.6 ± 0.1 ng/ml ($p < 0.001$), respectively compared to normal control group (Figure 4B). The extract (114 mg/kg) decreased ($p < 0.001$) the level of caspase 9 level in the hippocampus (64.67%) and prefrontal cortex (40.34%) (Figure 4B). Piracetam and Tacrine decreased ($p < 0.001$) the caspase 9 level in the hippocampus (59.23% and 53.43%, respectively) and prefrontal cortex (33.30% and 33.58%, respectively) (Figure 4B).

2.8. Effect of *Z. jujuba* extract on the neuronal alteration in the hippocampusand prefrontal cortex

The micro-architecture of the hippocampus of normal control rats shows intact neurons in CA1 and CA3 layers (Figures 5A and B) and regular thickness of dentate gyrus (Figure 5C). The normal density of neurons is also observed in the prefrontal cortex (Figure 5D). In the negative control, the thickness of layers (CA1 and CA3) and the density of neural cell bodies are reduced (Figures 5E and F). We also observe the presence of spongiosis (S), granulovacuolar degeneration (GVD) as well as chromatolysis (CH) in the CA3 layer (Figure 5F). Moreover, the dentate gyrus shows the presence of perivascular edema (PE) (Figure 5G). The prefrontal cortex shows a low density of neurons (Figure 5H). In rats treated with the extract (29 and 57 mg/kg), CA1 and CA3 layers showed a thickness comparable to the normal control group (Figures 5Q, R, U, and V). In the dentate gyrus, there is a decrease in perivascular edema (Figures 5S and W), while in the prefrontal cortex there is a normal density of neurons (Figure 5T and X).

2.9. Effect of *Z. jujuba* extract on the CA1 and CA3 neurons density of the hippocampus

Table 5 presents the effect of treatments on the density of CA1 and CA3 neurons. In Administration of scopolamine in distilled treated rats decreased the number of neurons in CA1 and CA3 to 61.1 ± 1.7 (44.51%) and 37.5 ± 1.5 (19.01%), respectively compared to the normal control group. The extract (29 mg/kg) prevented a decrease in the number of CA1 and CA3 neurons (Table 5).

Table 5: Effect of *Z. jujuba* extract on the CA1 and CA3 neurons density of the hippocampus

	DW+DW	SCO+DW	SCO+PI	SCO+TA	SCO+E29	SCO+E57	SCO+114
CA1	110.1 ± 3.1	61.1 ± 1.7[c]	89.3 ± 1.5***	74.1 ± 1.1*	91.7 ± 1.2***	85.3 ± 3.7***	78.5 ± 1.5**
CA3	46.3 ± 1.5	37.5 ± 1.5[a]	46.1 ± 1.1*	41.5 ± 1.5	45.5 ± 1.5*	45.1 ± 1.1*	40.5 ± 0.5

Each value represents the average ± ESM; n = 7-8. ap <0.05; cp <0.001 *vs* normal control. * p <0.05; ** p <0.01; *** p <0.001 *vs* negative control. s: Second; Lat.: Latency time; DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of *Z. jujuba* at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine; SCO + E29-E114: Test groups treated *Z. jujuba* extract; CA1: Cornus amonum 1; Cornus amonum 3.

3. Discussion

The purpose of this study was to assess the effect of *Z. jujuba* extract on cognitive impairments induced by scopolamine in rats, using Morris water maze and Y-maze paradigms. The morris water maze is used to assess long term spatial learning and memory in rodents [36]. During acquisition (4th day) and retention phases, injection of scopolamine in distilled water treated-rats increased the latency to find the platform. It also reduced the time spent in the target quadrant in the Morris water maze. Indeed, scopolamine induces amnesia by antagonizing muscarinic receptors of acetylcholine, which is the main neurotransmitter involved in the learning and memory process [37]]. These findings corroborate those of Li et al. [38] with increased time to reach the platform and decreased time spent in the target quadrant after scopolamine administration. However, during both phases, the extract of *Z. jujuba* and piracetam reversed these effects and suggesting an anti-amnesic effect. These results are in agreement with a study demonstrating that *Z. jujuba* ethanolic extract ameliorates cognitive impairments in rats [22]. The fact that the extract restores learning and memory impairments like piracetam, these findings might also suggest cholinergic blockade and emphasize its beneficial effect on long-term spatial memory dysfunctions [7, 22].

To determine the effect of the extract on short-term spatial memory, the Y-maze test was assessed. The Y-maze paradigm is used to assess short-term spatial memory in rodents [39]. In rats that received scopolamine and distilled water, the percentage of spontaneous alternation decreased. Surprisingly, the extract did not affect the percentage of spontaneous alternation. These results indicate that the extract does not interfere with short-term memory process dysfunctions, because Y-maze is sensitive to drugs that act on the working memory process [29, 40, 41]. This study gives an insight into the complex

mechanisms underlying short term memory dysfunction and reveals that this type of memory would not be affected in AD patients.

In AD, the increased deposition of beta-4-amyloid proteins (PβA) in the brain, induces the generation of reactive oxygen species (ROS) [42, 43]. These ROS generations are associated with cognitive impairments. In this study, administration of scopolamine-induced oxidative stress in the hippocampus and the prefrontal cortex. This event is characterized by an increase in the level of MDA, SOD, nitrite, and a decrease in GSH and total protein. Similar results were obtained by Isola et al. [44] and Ghasemi et al. [45]. Administration of the extract as well as piracetam or tacrine decreased SOD, nitrite, and MDA level, and increased those of GSH and protein total in both tissues. These data indicate that the extract possesses antioxidant activity [31]. This activity might be due to the presence of tannins, coumarins, phenols, triterpenes, and flavonoids evidenced in this study. Indeed, recent studies showed that the cytoprotective and neuroprotective effects of these molecules are strongly correlated to their antioxidant potential [46, 47]. These observations are in agreement with those of Yoo et al. [48], who showed that the neuroprotective effects of the methanolic extract of *Z. jujuba* fruit against ischemic damage in rodents result from the antioxidant properties of this essence. Taken together, these findings reveal therefore that the extract possesses a neuroprotective effect.

Neuroinflammation is not generally seen as a cause of AD, but rather as a result of AD [49]. Neuroinflammatory processes are known to be exacerbated by PβA and phosphorylation of tau protein deposit [50]. Therefore, drugs with anti-inflammatory activity could contribute to improving memory in patients with AD. Administration of scopolamine led to an increase in the level of TNF-α, IL-1β, and IL-6 in the hippocampus and prefrontal cortex of rats treated with distilled water [51]. The treatment of animals with the extract significantly decreased the level of these cytokines. These data suggest that the extract has anti-inflammatory properties [52]. The identified group of flavonoids in this plant might underlay this activity [53]. These results also suggest a neuroprotective effect and could explain in part its anti-amnesic effect.
During the pathogenic process in the brain of AD patients, it is well known that oxidative stress, as well as inflammation, could trigger apoptosis mechanisms [54]. Thus, inhibiting these mechanisms may hamper their impact on cognitive functions. In this study, scopolamine increased the level of caspases 3 and 9 in the hippocampus and the prefrontal cortex of distilled water-treated rats. These data are consistent with those of Demirci et al. [55]. In fact, administration of scopolamine induces the formation of PβA in the hippocampus and the prefrontal cortex [56]. PβA via the caspases cascade (mainly caspases 8, 9, and 3) leads to apoptosis of neuronal cells and contributes to the pathophysiology of AD [57]. Treatment of animals with the extract resulted in a significant decrease in caspases levels in the hippocampus and prefrontal cortex. These results suggest that the extract possesses anti-apoptotic properties and strongly support its aforementioned neuroprotective effect [58, 59]. These properties might involve some major secondary metabolites with anti-apoptotic activity such as flavonoids, triterpens and polyphenols [60, 61].

Analysis of the histological sections of scopolamine-treated rats showed a reduction in the number of neuronal cells in the CA1 and CA3 regions of the hippocampus. These results support those of Sayyahi et

al. [62]. The extract protected the hippocampus from neuronal degeneration in the CA1 and CA3 regions of the hippocampus. This protection was reflected by a preserved density of neurons in these regions. Given that CA3 and CA1 regions are involved in the learning and memory process [63], these results confirm its neuroprotective effect and subsequently explain its anti-amnesic effect. Consequently, this extract could be used to prevent only long-term memory dysfunctions or neurodegenerative processes in patients with AD, epilepsy, and Parkinson's disease. However, the lack of effect on short-term memory, despite evident antioxidant, anti-inflammatory, and anti-apoptotic in the prefrontal cortex highlights the complexity of short-term memory physiology. Further studies on isolated molecules need to be achieved to establish the exact mechanisms involved in the ant-amnesic and neuroprotective effects of the extract.

Conclusions

In sum, this study aimed to assess the anti-amnesic and neuroprotective effects of *Z. jujuba* leaves aqueous extract on the scopolamine model of AD in rats. Treatment with the aqueous extract protected animals from cognitive impairments. Analysis of possible mechanisms of action demonstrated that these effects are mediated in part by anti-oxidant, anti-inflammatory, and anti-apoptotic activities. These findings suggest that the extract possesses anti-amnesic and neuroprotective effects. This justifies its empirical use in the treatment of dementia in Cameroonian’s folk medicine. Furthermore, this extract could be used as an adjunct to treat diseases associated with spatial long-term memory impairments such as epilepsy, Alzheimer’s, and Parkinson’s diseases.

Abbreviations

ANOVA, analysis of variance; DW, distilled water, i.p., intraperitoneally; *p.o.*, *per os*; *Z. jujuba*, *Ziziphus jujuba*; SCO, scopolamine; S.E.M, standard error of the mean. IL-1β, interleukin-1beta; IL-6, interleukin-6; TNF-α, tumor necrosis-alpha; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase, AD, Alzheimer’s disease.

Declarations

Ethics approval and consent to participate

Animals procedures were carried out following the guidelines of the Institutional Ethics Committee of the Cameroon Ministry of Scientific Research and Innovation (Reg. no. FWA-IRD 0001954, 04/09/2006), which adopted the guidelines of the European Union on Animal Care (C.E.E. Council 86/609).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing of interest

There is no conflict of interest

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Authors' contributions

TD, AKK, ED designed the study, AKK and ED performed all behavioral studies, accomplished data analysis, and drafted the manuscript. TD, JK, FN, and SD critically revised the manuscript for important intellectual content. SL and DN helped in *in vivo* studies. All authors have read and approved the final manuscript.

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Figures

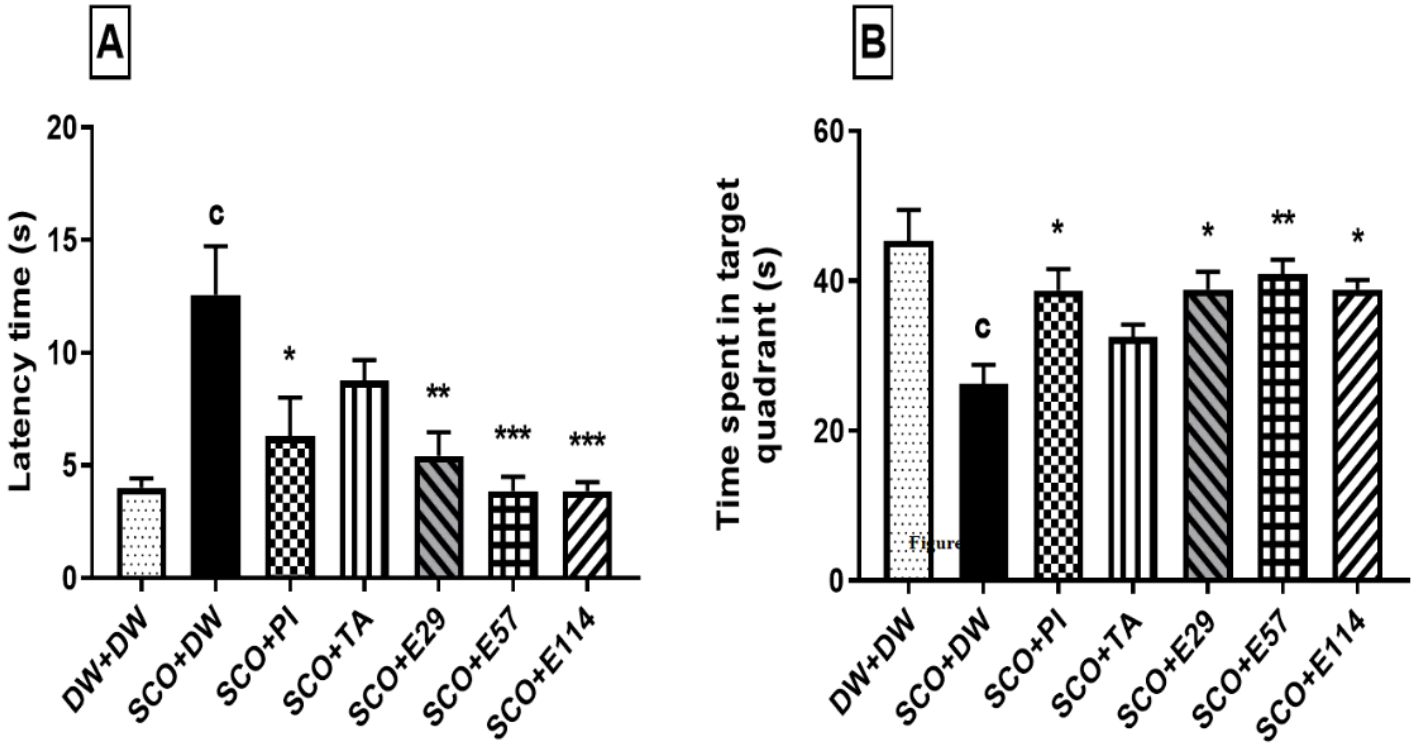

Figure 1

Effect Z. jujuba extract on the latency time to reach the platform (A) and the time spent in the target quadrant (B) in the Morris water maze during the retention phase.

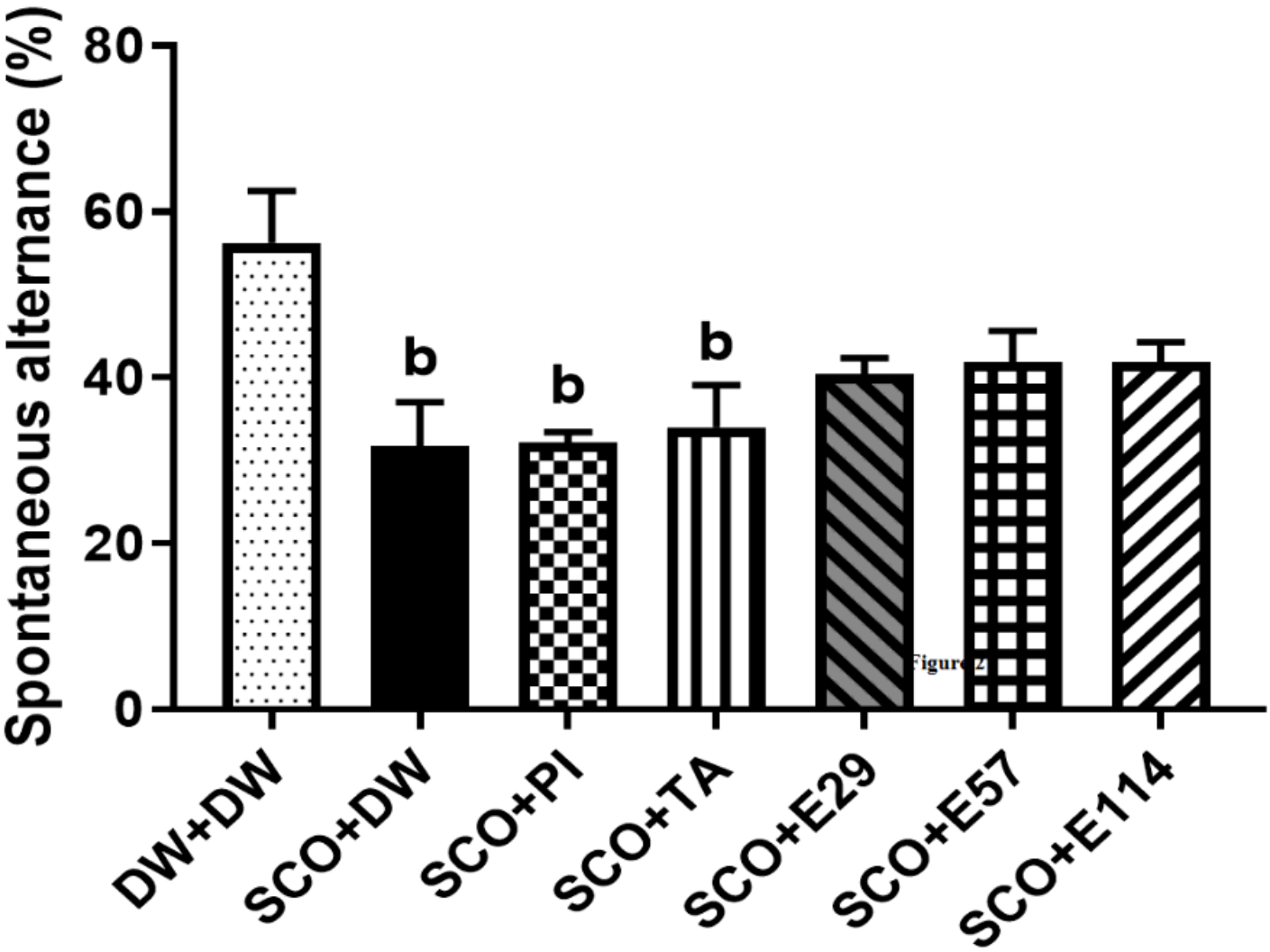

Figure 2

Effect Z. jujuba extract on the percentage of spontaneous alternation in the Y-maze. Each value represents the average ± ESM; n = 7-8. bp <0.01 vs normal control. DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of Z. jujuba at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine ; SCO + E29-E114: Test groups treated Z. jujuba extract.

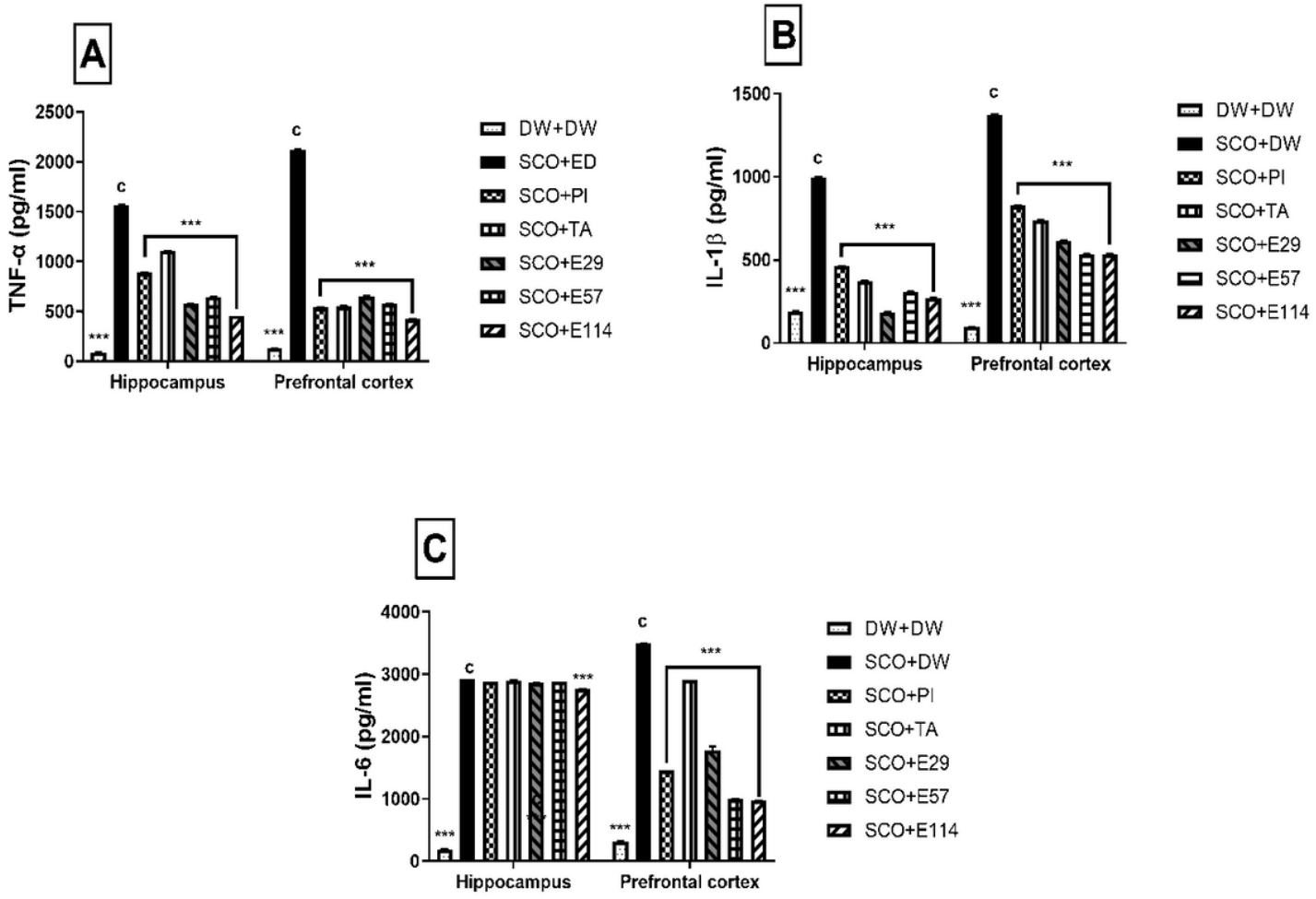

Figure 3

Effect of Z. jujuba extract on the pro-inflammatory markers in the hippocampus and the prefrontal cortex. Each value represents the average ± ESM; n = 7-8. bp <0.01 vs normal control. *** p <0.001 vs negative control. DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg); TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of Z. jujuba at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine ; SCO + E29-E114: Test groups

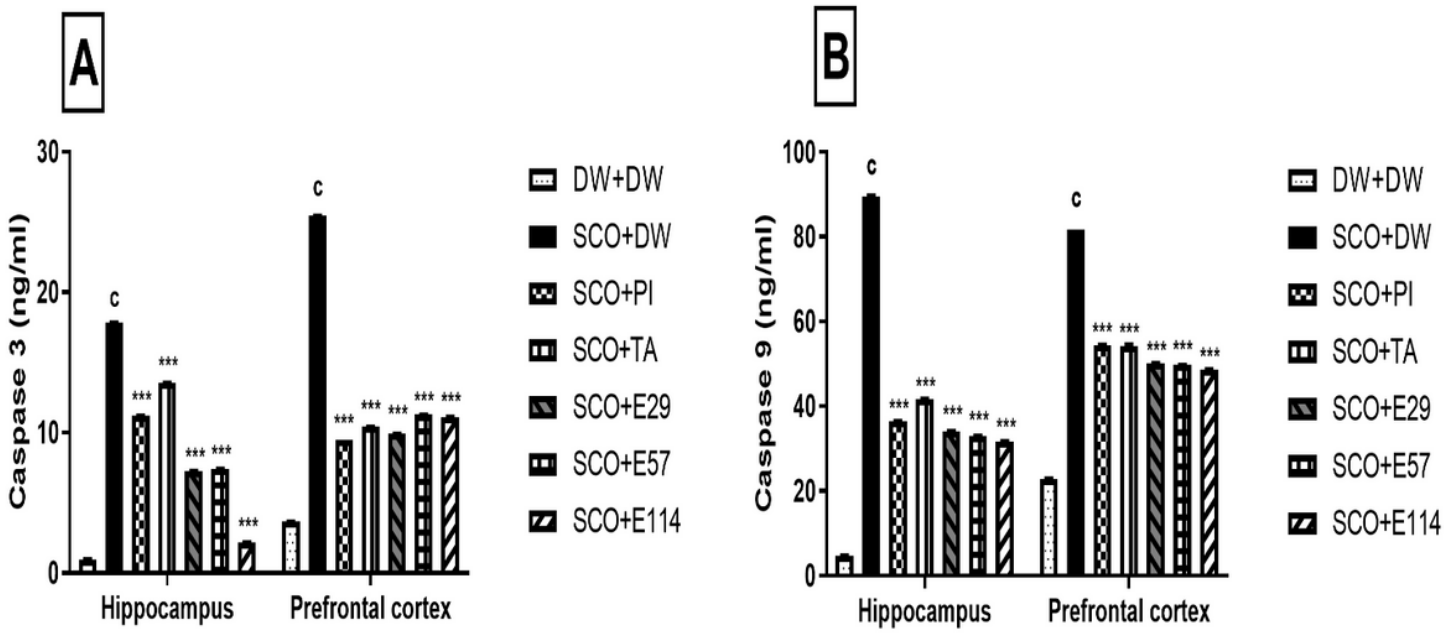

Figure 4

Effect of Z. jujuba extract on the apoptosis markers in the hippocampus and the prefrontal cortex. Each value represents the average ± ESM; n = 7-8. bp <0.01 vs normal control. *** p <0.001 vs negative control. DW: Distilled water (10 ml/kg); SCO: Scopolamine (1 mg/kg); PI: Piracetam (200 mg/kg) ; TA: Tacrine (10 mg/kg); E29; E57; E114: Aqueous extract of Z. jujuba at respective doses of 29, 57 and 114 mg/kg. DW + DW: Normal control group; SCO + DW: Negative control group; SCO + PI: Positive control group with piracetam; SCO + TA: Positive control group with tacrine; SCO + E29-E114: Test groups treated Z. jujuba extract.

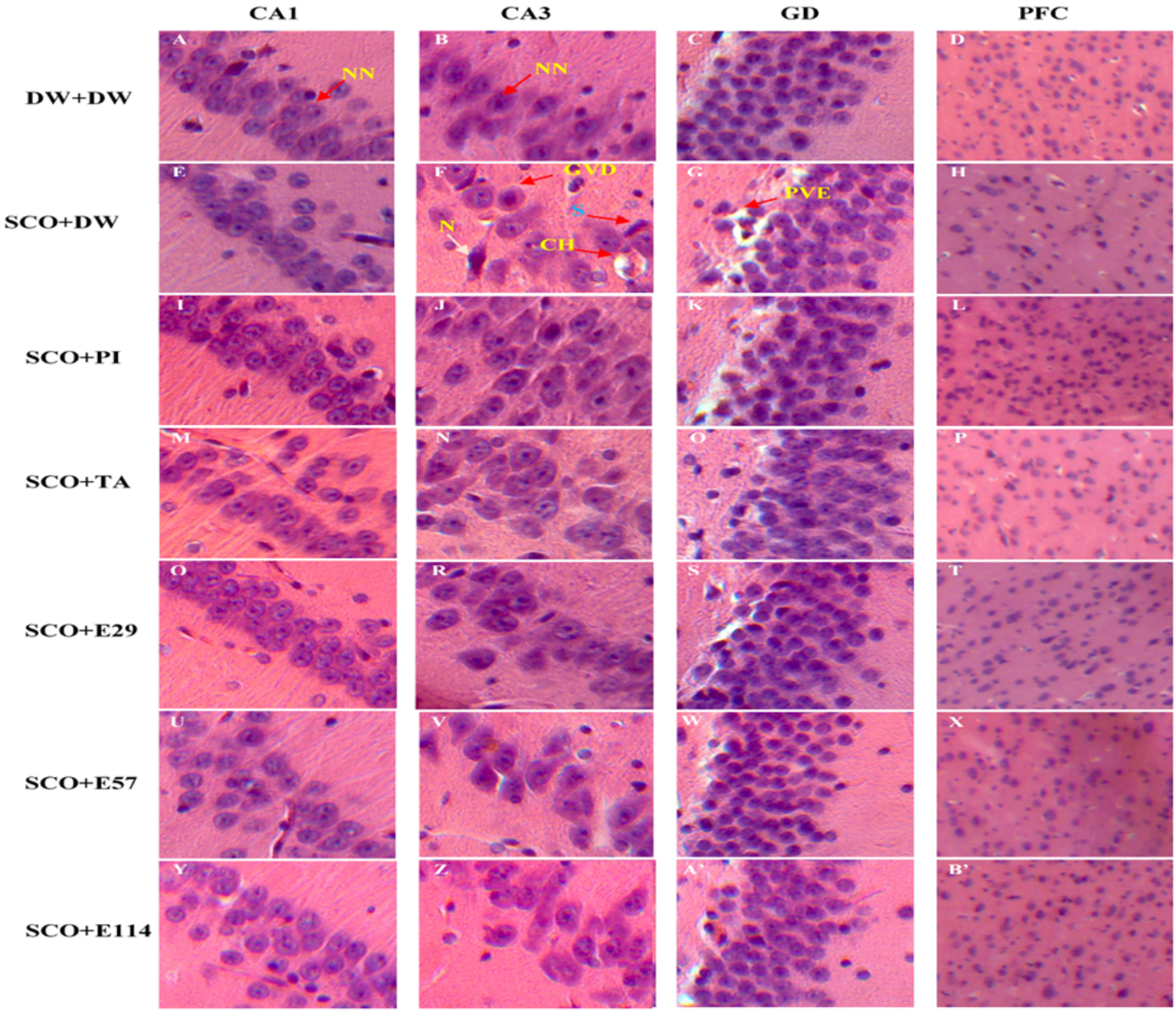

Figure 5

Microphotographs of the hippocampal tissue and prefrontal cortex sections after hematoxylin and eosin staining (x 250). Hippocampus (CA1, CA3, and GD) ; PFC: Prefrontal cortex; CA1 and 3: Cornus Ammon 1 et 3 ; DG: Dentate gyrus ; N: Neuron; NN: Normal neuron ; GVD: Granulovacuolar degeneration; S: Spongiosis; CH: Chromatolysis; PVE: Peri-vascular edema; DW : Distilled water (10ml/kg) ; SCO: Scopolamine (1 mg/kg) ; PI: Piracetam (200 mg/kg) ; TA: Tacrine (10 mg/kg) ; E29, E57 and E114: Aqueous extract of Z. jujuba at respective doses of 29 mg/kg, 57 mg/kg et 114 mg/kg. DW+DW: Normal control group; SCO+DW: negative control group; SCO+PI: Positive control group treated with piracetam; SCO+TA: Positive control group treated with tacrine; SCO+E29-E114: Test groups treated with the extract of Z. jujuba.