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Research article

The neuroprotective effect of *Xylopia parviflora* against aluminum chloride-induced neurotoxicity in rats

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ABSTRACT

Neurodegenerative disease such as Alzheimer's disease, are progressive disorders which has been linked to oxidative imbalance and associated perturbations characterised by loss of memory, cognition and cholinergic deficit. To date, cholinesterase inhibition and neuroprotection are the two major strategies in drug development. *Xylopia parviflora* (Annonacea family) is a spice consumed in Cameroon and has been used in traditional medicine to treat various pains. In this study, *X. parviflora* was evaluated on behavioural studies, ion homeostasis, cholinesterase inhibitory and antioxidant activities. Rats were exposed to aluminium chloride (75 mg/kg) during 60 days, and were treated with the extract of *X. parviflora* (150 and 300 mg/kg BW) and two drugs references (Donepezil and Curcumin). Behavioural parameters were assessed using the Morris-Maze test and the Open Field, followed by biochemical investigations, namely, cholinesterase enzyme activity (AChE and BChE), oxidative stress (NO, MDA, GSH level, SOD and Catalase activities) and ion homeostasis (Mg^{2+} and Ca^{2+} levels). $AlCl_3$ administration shows a decrease in learning and memory improvement during behavioural studies, significant alteration of the central cholinergic system characterised by an increase in AChE and BChE activities to 2.72 ± 0.002 mol/min/g and 5.74 ± 0.12 mol/min/g respectively, disturbance of ion homeostasis with an increase in Ca^{2+} level (25.68 ± 3.78 µmol/mg protein) and a decrease in Mg^{2+} level (15.97 ± 2.05 µmol/mg protein) and an increase in oxidative stress compared to the positive control group. Treatment with the different doses of *X. parviflora* increased memory and improved locomotion, improved cholinesterase activities, ion homeostasis and stabilized brain oxidative stress levels. The study suggests that *X. parviflora* could potentially be used for the management of some biochemical alterations associated with Alzheimer's disease. It could even be a good alternative to chemical drugs for neurotoxicity and memory enhancement.

1. Introduction

Neurodegenerative diseases (NDDs) are a group of disorders characterized by a progressive and persistent loss of neuronal activity. The origin of NDDs is multifactorial, involving a complex combination of genetic and non-genetic factors such as the aging process. NDDs affect a growing number of people worldwide [1]. Among the most common is Alzheimer's disease (AD). This disorder is characterized by impairment of learning, cognitive deficits, and behavioral disturbance. Currently, AD is the fourth leading cause of death in the elderly [2]. According to studies, aluminum is involved in the genesis of neurodegenerative diseases such as Alzheimer's disease through several mechanisms. Despite advances in knowledge of the pathophysiology of AD in recent years, cellular and molecular actors as well as causative factors of AD are still not identified with certainly [3]. Several studies have shown that increased exposure to aluminum leads to deregulation, toxicity and atrophy of the brain in rats. These deregulations are found in ionic homeostasis (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}), lipid profile markers (Cholesterol, HDL, LDL) and central cholinergic system (acetylcholinesterase and buturylcholinesterase) [4]. Apart from these, an association of reactive oxygen species (ROS) is also evident to cause the pathogenesis of AD.

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Aluminium is ubiquitous in the environment, being one of the most abundant elements in the earth's crust. Aluminium and aluminium compounds therefore occur naturally in ambient air and are a natural component of drinking water and many untreated foods such as fruits, vegetables and grains. Aside from its natural presence, aluminium is an environmental contaminant, due to anthropogenic releases associated with industrial processes [5, 6]. Therefore, Al induced oxidative stress which initiates the death of neuron through several cellular reactions like lipid peroxidation, protein oxidation, ROS formation, intracellular and mitochondrial calcium accumulation. According to the mechanism underlying aluminium neurotoxicty, Al induced damage of mitochondria and produced excessive ROS which cause DNA damage and finally apoptotic cell death [1]. In the central nervous system, neurotoxicty caused by metal is linked with the decrease of enzymatic activities, increased misfolded proteins and oxidative stress [5]. Research on treatments to control the progression of AD has led to the discovery of new therapeutic strategies such as neuroprotection [7]. Neuroprotective therapies must protect neurons against neurotoxins, free radicals and promote neuronal survival by regulating neurotrophic factors [8]. These therapies are particularly attractive because of their low toxicity, low cost and ability to target early disease changes. Many epidemiological studies also address the importance of nutrition in the development and management of neurodegenerative diseases [9]. People who benefit from the "Mediterranean diet" rich in olive oil, fatty fish and spices, are less exposed to age-related cognitive disorders [10, 11]. As fruits, spices and bark of edibles fruits used in the treatment of neurodegenerative diseases by their bioactive compounds, we have turmeric, *Piper methysticum*, *Evodia rutaecarpa*, *Baillonella toxisperma*, *Ginkgo biloba*, *Syzygium aromaticum, Carissa edulis*... used for their antioxidant, antiinflammatory and antiacetylcholinesterase activities [12].

Xylopia parviflora or 'Sedhiou pepper' is a spice widely consumed in Cameroon in the preparation of local dishes in West Region such as *'yellow soup'* and '*nkui*'. *Xylopia parviflora* is a plant of the Annonaceae family. Aromatic plants such as *Xylopia parviflora* are used as spices all over central and western Africa. It also used as components of herbal medicines for the treatment of skin infections, cough, bronchitis, dysentery, female sterility, fever and to prevent carcinogen-induced breast cancer [13]. In Cameroon, it can be found on the banks of rivers, in dense forest and in gallery forests. Fruits of *Xylopia parviflora* are used to make traditional Cameroonian soups. Previous studies conducted on aqueous and ethanolic extracts of *Xylopia parviflora* fruits have shown that they have good contents of polyphenols, alkaloids, proanthocyanins, tannins... antioxidant properties, antinociceptive effects *in vitro* [14]. The essential oil hydrodistilled from fruits was analysed for in vitro biological activities, namely cytotoxic, anti-inflammatory and antimicrobial [15]. Flavonoids, alkaloids, proanthocyanins, tannins isolated from this plant presented several pharmacological activities [16]. The nutritional properties of this spice (mineral composition, protein, amino acids, fats, lipids, bioactive compounds contents and their characterization), was determined [17, 18]. According to literature, *X. parviflora* possesses antioxidant and anti-inflammatory activities [18, 19]. Given the complexity of AD and the impressive number of excellent published works, modesty and simplicity are the order of the day. The identification of naturally occurring molecules as well as new therapeutic targets is a direction of interest for the treatment of these diseases [20, 21]. To our knowledge, the spice chosen for our study has not been the subject of any particular study concerning neural activity. In this work, we evaluated the neuroprotective potential of the combined filtrate of *X. parviflora* on neurotoxicity induced by aluminum chloride.

2. Methodology

2.1. Drugs and chemicals

Aluminum chloride, TNB (5- thio (2-nitrobenzoic acid)), DTNB (5,5-dithiobis (2-nitrobenzoic acid)), adrenaline, sulfanilamide, N-1-naphthylene diamine dichloride (NED), phosphoric acid, hydrogen peroxide (H_2O_2) hydrolyzing, potassium dichromate ($K_2Cr_2O_7$), trichloroacetic acid (TCA), thiobarbituric acid (TBA) are obtained from Sigma Ald Louis, MO, USA. Kits supplied by the manufacturers CHRONOLAB. Hydrochloric acid (HCl), sodium chloride (NaCl), distilled water and ethanol were used as solvents.

2.2. Plant material, harvesting and processing

The fruits of *Xylopia parviflora* were purchased at the Bafoussam market (West Cameroon). The identification was done by the Cameroon National Herbariumin, Yaounde (in comparison with specimen N° 2018 Herbier 9203/SRF/CAM). The fruits were washed, dried and crushed to obtain powder which was kept in the opaque flasks. This powder has been used to prepare the extracts.

2.3. Experimental animal

Thirty (30) female rats of Wistar strain bred in our laboratory (Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, Faculty of Science, University of Yaoundé 1) and aged of 15 months were used in this study. They were weighing between 200 and 220 g and maintained in cages under controlled conditions (25 °C) with free access to food and water. Animals were treated in accordance with the guidelines of the ethical committee of the University of Yaounde 1 and the Guide of the Care and Use of Laboratory Animals (8^{th} edition). For this study, we reduced the number and minimize suffering of animals and carried out in accordance with the national principles of laboratory animal care (number FWA-IRB00001954) and the Ethic Committee of the Faculty of Sciences of the University of Yaounde 1 (N°31/02370/UY1/D/FS/VD-RC).

2.4. Preparation of extracts

The fruit powders obtained were subjected to two types of extraction: hydroethanolic and ethanolic. Concerning hydroethanolic extraction, the powders were dissolved in a mixture water-ethanol 1:4 (W/V). After homogenization, the mixture was macerated for 48 h at room temperature and the supernatant was then filtered with Whatman filter paper N°2.

Concerning ethanolic extraction, the residue obtained after hydroethanolic extraction was dissolved in ethanol in the proportions 1:4 (W/V). The mixture macerated for 24 h at room temperature and the supernatant was then filtered.

The two filtrates (hydroethanolic and ethanolic) obtained were mixed to form the combined filtrate and it was later concentrated at 50 °C for 72 h. The mixture obtained after concentration was stored at 4 °C for further handling.

2.5. Experimental design

Memory impairment was induced in rats by oral administration of aluminium chloride (75 mg/kg BW) and subsequent evaluation of behavioural and biochemical effects were carried out. The experimental design 5× 6×6 was adopted, with 5 representing the number of animal per group, 6 represents the number of group while 6 stands the number of treatment. Six groups of five rats each were formed and divided as follows: a negative control group received distilled water daily and orally for 60 days, a positive control group received 75 mg/kg aluminium trichloride ($AlCl_3$) daily by oesophageal gavage and 4 other groups received 150 and 300 mg/kg/BW/day respectively of a combined filtrate extract of *X. parviflora*, a cholinergic reference drug (Donepezil) 5 mg/kg/BW/day and an antioxidant reference compound (Curcumin) 100 mg/kg/PC/day, 45 min after receiving $AlCl_3$. The extract doses of 150 and 300 mg/kg body weight were selected after pre-experiment on acute and subacute toxicity study of *X. parviflora*. The ability of all these selected drugs to penetrate into Blood-Brain Barrier was

demonstrated in the literature [22, 23, 24, 25]. The extracts and reference compounds were prepared in distilled water and administered via the oesophagus using a probe. The experimental scheme is summarised in Table 1 below and the duration of exposure (60 days) was chosen based on the work of Nampoothiri *et al.* [26].

Behavioural parameters were performed at the 20th and 40th days of the experimental period, rats were fasted 12 h prior to sacrifice. Rats were weighed and sacrificed by cervical dislocation; the brains of the rats were collected, weighed and used to prepare homogenates. Before and after sacrifice, two main neurological disorders were assessed: cognitive impairment (behavioural tests, brain atrophy, cholinergic system, ion homeostasis) and oxidative stress.

2.6. Evaluation of cognitive function

The following behavioural tests were used:

Morris Maze Test [27]: The experimental device of the Morris pool test consisted of a circular enclosure 160 cm in diameter and 60 cm in height, filled to half height with water 22 ± 2 °C. The water was made opaque by adding milk powder. The platform, consisting of a cylinder (11 cm diameter) made of transparent plastic, was immersed 1 cm below the surface in order to be invisible to the animal. The pool was placed in a room with different distal indices (shelves with cages, painted pipes, ventilation, geometric shapes, etc.) that could allow the animal to find its way around the enclosure and to memorize the position of the platform in relation to an allocentric frame of reference. The rat's task was to learn how to reach the platform as directly as possible. The path taken by the animal was filmed using a video camera. The following parameters were measured: the time spent in the target quadrant, the latency time, the number of entries in the target quadrant and the distance travelled in the target quadrant.

Object Recognition: Open Field test [28, 29]: Locomotor activity was measured in the Open Field test, thanks to a device frequently used in experimentation. The device used was a square Plexiglas enclosure with a side length of 1 m and a height of 50 cm. It was divided into 2 parts of the same area: 1 central part and 1 peripheral part. The central part was used as a starting point for the animals for each test. Locomotion in the open field was evaluated by recording the total distance corvered; the time spent standing still (Downtime) and the number of turn arounds.

2.7. Sacrifice of rats and preparation of brain homogenates

After the rats were sacrificed by cervical dislocation, the brains were immediately removed and placed in ice-cold isotonic saline for few minutes. The brains were then weighed and cut into small pieces and homogenised to 10% in phosphate buffer (0.1 mol/L; pH 7.4) containing 0.3 mol/L sucrose and 0.08 mol/L potassium chloride. The homogenates were then centrifuged at 6000 rpm for 10 min at 4 °C. They were used to estimate ionic homeostasis (Ca^{2+}, Mg^{2+}), antioxidant parameters (malondialdehyde (MDA), nitrite oxide (NO), catalase (CAT), superoxide dismutase (SOD), Glutathione (GSH), total protein and cholinesterase enzyme inhibitory activity (AChE and BChE).

Cerebral atrophy: Brain atrophy was assessed by calculating the relative weight of the brain using the formula:

$$RBW_{(g/100g)} = 100 \text{ x (Brain weight (g) / Body weight (g)}$$

Determination of AChE/BChE inhibitory activity [30]: The method was based on the measurement of thiol groups by monitoring the concentration of yellow coloured TNB (5- thio (2-nitrobenzoic acid) formed by reduction of DTNB (5,5-dithiobis (2-nitrobenzoic acid). For the enzyme source, the rat brain (AChE)/rat blood (BChE) was homogenized in a homogenizer with 5 mL of a homogenization buffer [10 mM Tris–HCl (pH 7.2), which contained 1 M NaCl, 50 mM $MgCl_2$ and 1% Triton X-100], and centrifuged at 1000 rpm for 30 min. Introduce 1 mL of phosphate buffer, 100 μL of DTNB, and 25 μL of acetylthiocholine/buturylcholine iodide. This forms the blank with which the zero was adjusted. For the samples to be determined, 10 μL of the homogenate diluted three times was added and the absorbance was read at 412 nm. Each test was repeated 3 times and the average of the 3 values was taken. The progressive increase in absorbance was recorded for 5 min.

Evaluation of ionic homeostasis in brain homogenates: Calcium ions (Ca^{2+}) were measured following the kit Sigma-Aldrich protocol (Number MAK022) and Magnesium ions (Mg^{2+}) were measured following the kit Sigma-Aldrich protocol (Number MAK026).

2.8. Oxidative stress markers and total proteins assay

Malondialdehyde: the protocol described by [31] was adopted with slight modifications. Carbonyl compounds such as malondialdehyde react with thiobarbituric acid (TBA) to give pink chromophores absorbing at 530 nm. One hundred microlitres of homogenate or 0.9% NaCl (blank), 250 μL of trichloroacetic acid (TCA) 20% and 400 μL of TBA 0.67% were added to the glass screw-top tubes (blank and test) and sealed. The mixture was heated in a water bath at 100 °C for 15 min and then cooled in a cold water bath for 30 min. The tubes were left open to allow the gases formed during the reaction to escape. They were then centrifuged at 1500 rpm for 5 min and the absorbance of the supernatant was read at 532 nm against the blank. The concentration of MDA were expressed in mmol/g.

Reduced Glutathione (GSH) [32]: The method was based on the measurement of thiol groups by monitoring the concentration of yellow coloured TNB (5-thio-2-nitrobenzoic acid), formed by reduction of DTNB (5,5-dithiobis (2-nitrobenzoic acid). One hundred (100) μL of homogenate and 900 μL of Ellman's reagent were respectively introduced into blank and test tubes. After homogenisation, the mixture was incubated at room temperature for 30 min. Optical densities were read at 420 nm against the blank containing 900 μL of reagent solution and 100 μL of NaCl (0.9%) incubated under the same conditions. The concentration of reduced GSH in the brain tissues were expressed as millomoles per gram tissue (mmol/g tissue).

Catalase Activity [33]: The method was based on the fact that catalase present in the homogenate reduces hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). H_2O_2 not reduced by catalase binds to potassium dichromate to form a blue-green precipitate of unstable perchloric acid. This was then decomposed by heat to form a green complex that absorbs at a wavelength of 570 nm. The activity of catalase was proportional to the optical density and was determined by the

Table 1. Experimental design and drug doses in different groups of rats.

Groups	Name of groups	Compounds and drugs administered
NC	Normal Control	Distilled water
PC	Positive Control	$AlCl_3$ (75 mg/kg BW/day) + Distilled water
XP 150	Test group 1	$AlCl_3$ (75 mg/kg BW/day) + Combine filtrate of *Xylopia parviflora* (150 mg/kg BW/day)
XP 300	Test group 2	$AlCl_3$ (75 mg/kg BW/day) + Combine filtrate of *Xylopia parviflora* (300 mg/kg BW/day)
Donep5	Standard treatment cholinesterase	$AlCl_3$ (75 mg/kg bw/day) + Donepezil (5 mg/kg BW/day)
Cur	Standard treatment antioxidant	$AlCl_3$ (75 mg/kg BW/day) + Curcumin (100 mg/kg BW/day)

calibration curve. The activity was expressed as mmoles of H_2O_2 decomposed per mg protein.

Superoxide dismutase activity: method described by [34] was adopted. The method was based on the fact that SOD present in a sample inhibits the oxidation of adrenaline to adrenochrome. An aliquot of 0.2 mL of haemolysate was introduced into 2.5 mL of carbonate buffer (pH 10.2) to equilibrate the spectrophotometer. The reaction was then started by adding 0.3 mL of freshly prepared adrenaline to the mixture. After homogenisation by inversion the final mixture was read at 480 nm every 30 s until 150 s to follow the increase in absorbance. SOD activity was reported as units per mg of protein.

Ntrite oxide levels: This reaction was based on the diazotization reaction described by [35]. It described the chemical reaction using sulphanilamide and N-1 Naphthylenediamine dichloride (NED) in an acid medium (Phosphoric acid). This system detects the nitrite formed (one of the stable and non-volatile primary compounds of NO degradation) in biological lipids. To 100 μL of sample, 100 μL of a sulfanilamide solution was added. The mixture was incubated for 5–10 min at room temperature and protected from light. After incubation, 100 μL of N-1-naphthylenediamine dichloride solution was added and incubated again for 5–10 min at room temperature in the dark. The absorbance was read at 540nm against the blank. The concentration of NO was expressed in mmol/g.

Total Proteins assay: It was done according to [36]. Briefly, the reaction occurs under alkaline pH conditions with proteins using Biuret reagent containing copper sulfate, potassium iodide and sodium potassium tartrate. The protein and Biuret reagent form complexes with maximum absorbance at 540 nm. Brain homogenate were added to 3 mL of Biuret reagent and incubate at room temperature for 30 min. The absorbance of mixture was readed at 540 nm. The intensity of the staining was correlated to the quantity of proteins present in the sample and expressed using a standard curve of bovine serum albumin at 3 mg/mL.

ATPases Activities Assay: This was prepared according to [37]. The method consisted of the quantification of phosphate release after cleavage in the presence of enzyme and ATP using ouabain or not. Briefly, in the first tube, 350 μL of assay buffer containing NaCl 100 mM, KCl 10 mM, MgCl 10mM EGTA 0.1 mM in histidine HCl-Tris 25 mM pH 7.5 was mixed with 40 μg of homogenate protein from rat's brain. For the same animal and in the same condition, another test tube was made but 1.5 mM of ouabain was added. All the tubes were pre-incubated at 37 °C for 10 min, and the reaction was started by adding 50 μL of ATP. After that, 5 mM of Na_2-Tris was added for a final volume of 500 μL. After 1h, the reaction was stopped by adding 500 μL of 15% (w/v) of cold trichloroacetic acid solution. The inorganic phosphate present in solution was quantified by the colorimetric method of [38] and was used to estimate the activity of ATPase according to a standard curve of sodium monobasic phosphate. The activity of Na^+, K^+-ATPase was determined by subtracting the sensitive activity of ouabain (Mg^{2+}-ATPase activity) from the total activity of ATPase. The activity of ATPases was expressed as μmol Pi/mg protein/hour.

2.9. Histopathological studies

Histopathological evaluation of Ammon's horns of the hippocampus of rat brains was performed. After behavioural studies, the brains were excised and immediately fixed in 10% buffered formalin. The hippocampus was sectioned from the brain, which was embedded in paraffin after being dehydrated in alcohol. Five-micrometer thick serial histological sections were obtained from the paraffin blocks by using microtome and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.

2.10. Statistical analysis

Data were analysed using GraphPad Prism software version 5.0. The data was presented as the mean ± SEM. For normally distributed data, comparison among the different groups was done using oneway ANOVA followed by the Tukey multiple comparison test (SPSS, Inc, IBM Corporation, Chicago, USA). The $AlCl_3$ group was analysed compared to the control normal group, and the groups receiving the extract or treatment were compared to the $AlCl_3$ group. Results were significant at $p < 0.05$. The Excel spreadsheet (version 2013, Microsoft Corporation by Impressa Systems, Santa Rosa, California) was used to plot the graphs.

3. Results

3.1. Effects of X. parviflora on aluminium chloride-Induced Memory Impairment in the Morris Maze Test

Figures (1A, 1B, 1C and 1D) represent respectively, the number of entries, the time spent, the latency and the distance covered in the target quadrant. Aluminium resulted in a memory deficit marked by a decrease in the number of entries, time spent and distance travelled in the target quadrant, combined with an increase in latency, when comparing positive control (PC) to negative control (NC). The deficit was more pronounced over time. The extract improved memory with an increase in the number of entries, time spent and distance travelled in the target quadrant, combined with a decrease in latency with time. At the dose of 300, the effect of extracts was more pronounced over time. The extract at 300 was more effective than the 2 references.

3.2. Locomotion activity in open field

Figures (2A, 2B and 2C) represent respectively, the distance covered, the number of turn arounds and the downtime. Analysis of our results shows, that locomotor activity was significantly ($p < 0.05$) reduced or even disrupted in all groups receiving $AlCl_3$ compared to Normal Control; this was reflected in the decrease in total distance covered, the number of turnarounds, the number of entries to the centre and the increase in downtime. In tests and references groups, compared to the positive control (PC), there was an increase in the distance covered, the number of turn arounds and a decrease in the downtime as shown in the graphs below.

3.3. Effect of treatment on brain atrophy and cholinesterase activities

Table 2 represents the effect of *X. parviflora* on brain atrophy and cholinesterase activities. In Table 2, the decrease (no significant) in brain mass of untreated rats ($AlCl_3$) was first compared to that of normal control rats and also to that of rats undergoing treatment after intoxication (0.0079 vs 0.0081). The extract (*X. parviflora*) showed the ability to limit this cerebral atrophy compared to the $AlCl_3$ group (0.0082 and 0.0081 vs 0.0079 at 150 and 300 mg/kg BW respectively) and more effectively compared to the reference drugs used.

In the same Table, $AlCl_3$ increases the activity of cholinesterases ($p < 0.05$) in the brains of rats compared to the normal control group (2.72 vs 1.30 mol/min/g for AChE and 5.74 vs 4.31 mol/min/g for BChE). Treatment with different doses of *X. parviflora* extract shows a memory enhancer effect by reducing cholinesterases activities ($p < 0.05$) compared to the group receiving only $AlCl_3$ (1.95 and 1.40 vs 2.72 mol/min/g for AChE; 4.58 and 4.48 vs 5.74 mol/min/g for BChE at doses of 150 and 300 mg/kg B.W respectively). There is no significant difference between Donepezil and extracts of *X. Parviflora* in the inhibition of cholinesterases activities.

3.4. Effect of the extract on ionic homeostasis

Table 3 below presents the effect of extracts of *X. parviflora* on the cerebral concentrations of calcium and magnesium in female rats. It appears that the lonely administration of $AlCl_3$ revealed an increase in the concentration of Ca^{2+} ions in the brain and a decrease in Mg^{2+} in PC group (25.68 ± 3.70 and 15.97 ± 2.05 μmol/mg protein respectively) compare to NC group (20.84 ± 2.28 and 28.91 ± 1.01 μmol/mg protein

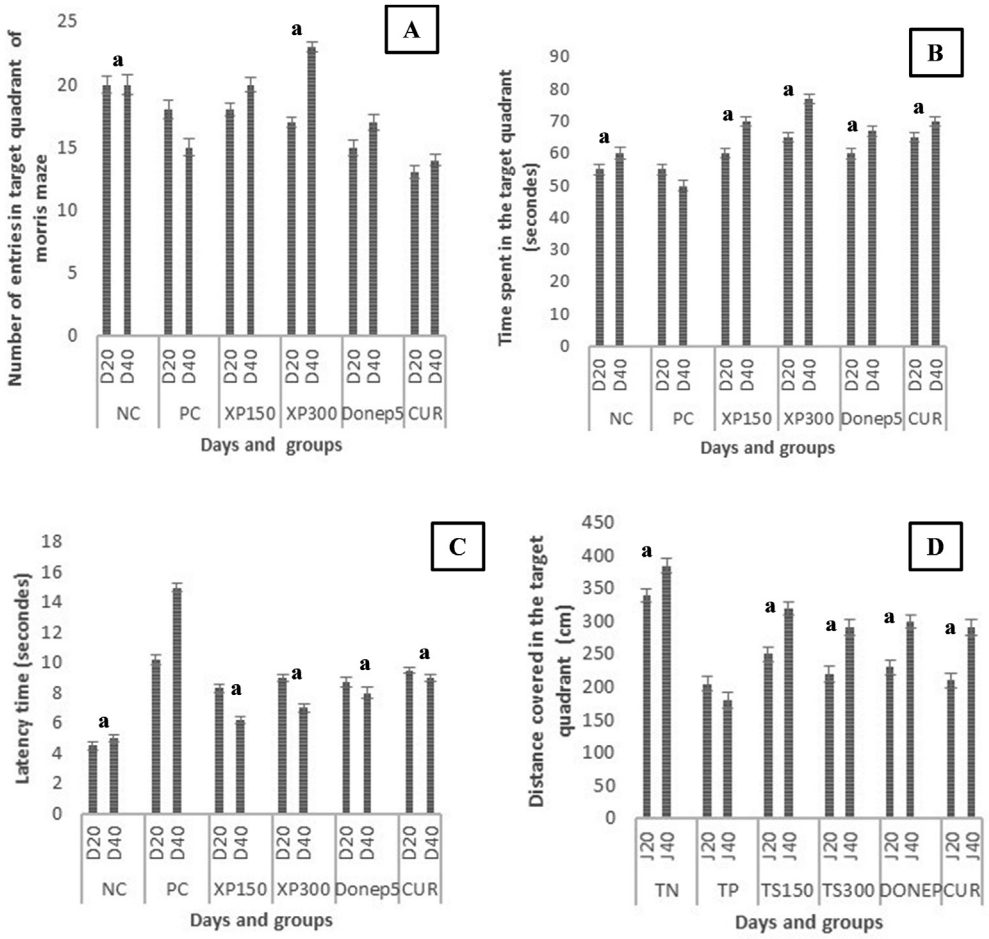

Figure 1. Effects of *X. parviflora* on aluminium chloride-Induced Memory Impairment in the Morris Maze Test. Animals were treated without or with $AlCl_3$ (75 mg/kg/BW *per os*), or cotreated with $AlCl_3$ (75 mg/kg/BW *per os*) and extract of *Xylopia parviflora* (150 and 300 mg/kg/BW/day *per os*) or reference compounds (Donepezil, 5 mg/kg/BW/day *per os*) and (Curcumin, 100 mg/kg/PC/day *per os*) for 60 days. Behavioural parameters (memory impairment) were performed during and at the end of the experimental (A): Effect of extract on number of entries in the target quadrant of the Morris Maze (B): Effect of extract on time spent in the target quadrant of the Morris Maze (C): Effect of extract on latency to enter in the target quadrant of the Morris Maze (D): Effect of extract on distance covered in the target quadrant of the Morris Maze. [a]*p < 0.05 vs positive control (PC).*

respectively). However, the co-administration of $AlCl_3$ and *X. parviflora* extracts has improved ionic homeostasis by significantly decreasing Ca^{2+} (20.75 ± 3.56 and 15.80 ± 3.20 µmol/mg protein) and increasing Mg^{2+} (25.85 ± 2.02 and 30.90 ± 1.15 µmol/mg protein) levels at dose of 150 and 300 mg/kg BW respectively. Donepezil and Curcumin treatments also significantly increased the Mg^{2+} and decreased Ca^{2+}concentrations compared with positive control group. Treatments with reference drugs (Donepezil and Curcumin) also lowered the brain Ca^{2+}concentration better than extract for Donepezil ($p < 0.05$) and increased Mg^{2+} level less than extract ($p > 0.05$).

3.5. Antioxidant status assessment

Table 4 represents the effect of *X. parviflora* extracts on cerebral antioxidant status in the female rats. It appears that the administration of $AlCl_3$ induces oxidative damage in animals. Like high concentrations of prooxidant status markers (MDA and NO) and a significant decrease ($p < 0.05$) in activities of antioxidant enzymes (CAT, SOD and GSH) in PC compared to NC. Treatment with extracts of *X. parviflora* resulted in significant reduction ($p < 0.05$) of NO (4.85 and 4.6 mmol/g at 150 and 300 mg/kg respectively vs 7.52 mmol/g in PC) and MDA (70.99 and 64.87 mmol/g at 150 and 300 mg/kg respectively vs 110.16 mmol/g in PC). At the level of MDA, this reduction was higher ($p < 0.05$) with extracts 300 mg/kg BW. In addition, the extracts have been ameliorated enzymatic antioxidant status ($p < 0.05$). The similar results have been observed with curcumin 100 mg/kg BW but at the level of CAT activity curcumin 100 mg/kg BW presented a significant increase of CAT activity compared to both doses of extracts.

3.6. Effect of treatment on ions contents and ATPases activities

The results showed a significant decreases in Na^+, K^+-ATPase, Mg^{2+}-ATPase (2.4; 3.7 µmol Pi/h/mg protein) activities, in Mg^{2+} contents (15.91 µmol/mg protein) and significant increase in Ca^{2+} contents (25.68 µmol/mg protein) in positive control rats compared to normal control rats (3.8; 5.5; 28.91 and 20.84 respectively). The rats treated with the extract (150 and 300 mg/kg bw) showed a significant increase in Na^+, K^+-ATPase (3.5; 4.1 µmol Pi/h/mg protein) and Mg^{2+}-ATPase (5.9; 6.1 µmol Pi/h/mg protein) activities and concomitant increases in Mg^{2+} levels (25.85; 30.90 µmol/mg protein) and decrease in Ca^{2+} (20.75; 18.80 µmol/mg protein) levels. Donepezil and Curcumin administration significantly increased Na^+, K^+-ATPase, Mg^{2+}-ATPase activities and the Mg^{2+} level and decreased Ca^{2+} contents compared to positive control (Table 5).

3.7. Effect of treatment on microarchitecture of the hippocampus

The results of the study of histological sections of the rat brain after aluminum chloride intoxication are shown in Figure 3. The microarchitecture of the hippocampus showed in the normal control (NC), a normal structure with intact looking neurons in this selected brain layer. Compared to the normal control, the positive control showed a pathological change in the hippocampus, marked by a decrease in the number of neuronal cells in the same layer. The extract at different doses of 150 and 300 mg/kg, as well as the reference drugs Donepezil and Curcumin resulted in an improvement of the hippocampal structure, close to that of the normal control.

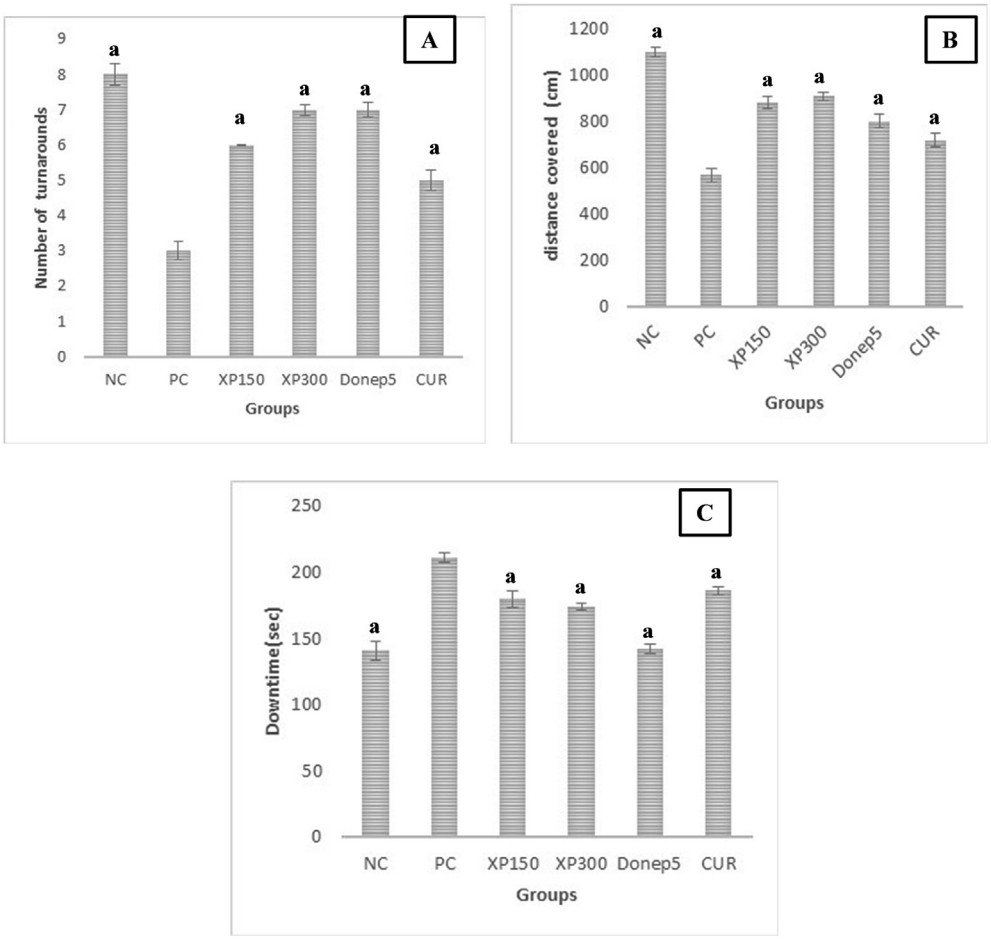

Figure 2. Effects of *X. parviflora* on aluminium chloride-Induced impairment in the locomotion activity in Open Field. Animals were treated without or with $AlCl_3$ (75 mg/kg/BW *per os*), or cotreated with $AlCl_3$ (75 mg/kg/BW *per os*) and extract of *Xylopia parviflora* (150 and 300 mg/kg/BW/day *per os*) or reference compounds (Donepezil, 5 mg/kg/BW/day *per os*) and (Curcumin, 100 mg/kg/BW/day *per os*) for 60 days. Behavioural parameters (locomotion impairment) were performed during and at the end of the experimental (A): Effect of extract on distance covered (B): Effect of extract on number of turnarounds; C: Effect of extract on down time. $^{a}p < 0.05$ *vs positive control (PC).*

4. Discussion

In this study, we evaluated the protective effects of *Xylopia parviflora* extract against aluminum chloride-induced neurotoxicity in Wistar strain rats. This animal experimental model is commonly used to assess the neuroprotective effects of a substance or plant that could have beneficial effects against neurodegenerative diseases [39]. Neurodegenerative diseases such as Alzheimer's disease are characterized by progressive pathological changes in the brain that result in clinical signs of declining cognitive (memory) abilities, functional abilities and finally physical changes. Pathological changes in the Alzheimer brain include the deterioration and loss of neurons (nerve cells) leading to brain atrophy [1, 2, 40]. We studied the neurotoxicity of aluminum related to cellular damage via: oxidative stress by measuring some markers of oxidative stress and deregulation of cognitive decline (behavioural tests, cerebral atrophy, ionic homeostasis and cholinesterase inhibition) in rats. In a first step, we evaluated the effects of the spice on memory and locomotion disorders following neurodegeneration. Administration of aluminium chloride caused memory and locomotion disorders, however, administration of the extracts prevents memory deficiency by reducing the dwell time, latency and increasing the number of inputs, distance travelled in the target quadrant. The decrease in latency and the significant increase in the number of entries and time spent in the target quadrant refer to improved memory function [41, 42]. At the same time, the increase in the number of entries in the target quadrant over time shows that there was an increase in exploration. Furthermore, the maintenance of memory capacity and the reduction of stress by the presence of food reinforcers are favourable for a good functioning of the memory. Similar results were obtained by Farshchi *et al.* [43]. Studies have shown that toxicant-induced stress can impair memory according to Li *et al.* [44]. Therefore, it can be said that the combined filtrate of *X. parviflora* could

Table 2. Effect of *Xylopia parviflora* on brain atrophy and cholinesterase activities in female rats treated with aluminum chloride for 60 days.

Groups	RBW	AChE (mol/min/g)	BuChE (mol/min/g)
NC	0.0081 ± 0.0002	1.30 ± 0.03[a]	4.31 ± 0.02[a]
PC	0.0079 ± 0.0003	2.72 ± 0.02	5.74 ± 0.12
XP150	0.0082 ± 0.0005	1.95 ± 0.01[a]	4.58 ± 0.07[a]
XP300	0.0081 ± 0.0001	1.40 ± 0.03[a]	4.48 ± 0.21[a]
Donep5	0.0080 ± 0.0001	1.25 ± 0.02[a]	4.40 ± 0.11[a]
Cur 100	0.0080 ± 0.0001	1.62 ± 0.03[a]	4.75 ± 0.04[a]

NC: Normal Control; PC: Positive Control; RBW: Relative brain weight; Donep: Donepezil; Cur: Curcumin. The values are expressed as mean ± sem. $^{a}p < 0.05$ vs positive control (PC).

Table 3. Effect of *Xylopia parviflora* on Mg^{2+} and Ca^{2+} concentrations in the brains of rats.

Groups	Calcium (µmol/mg protein)	Magnesium (µmol/mg protein)
NC	20.84 ± 2.28[a]	28.91 ± 1.01[a]
PC	25.68 ± 3.78	15.97 ± 2.05
XP150	20.75 ± 3.56[a]	25.85 ± 2.02[a]
XP300	15.80 ± 3.2[b]	30.90 ± 1.15[a]
Donep5	14.48 ± 4.24[b]	27.89 ± 2.02[a]
Cur 100	18.97 ± 3.56[a]	23.87 ± 1.01[a]

NC: Normal Control; PC: Positive Control; Donep: Donepezil; Cur: Curcumin. The values are expressed as mean ± sem. $^{a}p < 0.05$; $^{b}p < 0.01$ vs positive control (PC).

Table 4. Effect of *X. parviflora* extract on cerebral antioxidant status in the female rats.

Groups	NO (mmol/g)	MDA (mmol/g)	Glutathione (mmol/g)	SOD (unit/mg protein)	Catalase (mmol H_2O_2/mg protein)	Protein (g/dL)
NC	4.57 ± 0.52[a]	60.70 ± 5.10[a]	5.16 ± 0.48[a]	35.72 ± 5.02[a]	290.56 ± 14.34[a]	5.96 ± 1.23
PC	7.52 ± 0.39	110.16 ± 8.13	2.51 ± 0.20	26.86 ± 6.29	115.25 ± 10.65	5.80 ± 0.55
XP150	4.85 ± 0.79[a]	70.99 ± 6.11[b]	5.20 ± 0.57[a]	32.93 ± 4.97[a]	274.36 ± 18.78[a]	6.12 ± 0.23
XP300	4.60 ± 0.49[a]	64.87 ± 3.11[a]	5.49 ± 0.1[a]	36.85 ± 3.21[a]	325.00 ± 18.89[c]	5.96 ± 0.78
Donep5	5.05 ± 0.50[a]	85.48 ± 5.14[b]	4.6 ± 0.13[b]	28.85 ± 3.33	251.44 ± 32.55[a]	6.29 ± 1.04
Cur 100	4.59 ± 0.90[a]	60.92 ± 7.19[a]	5.67 ± 0.22[a]	37.45 ± 4.5[a]	423.60 ± 10.14[c]	6.04 ± 0.11

NC: Negative Control; PC: Positive Control; Donep: Donepezil; Cur: Curcumin; NO: Nitric oxide, MDA: Malondialhyde, SOD: SuperOxide Dismutase. The values are expressed as an average ±standard error on the average. [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ vs positive control (PC).

Table 5. Effect of *X. parviflora* extract on ATPases Activities in Brain.

Groups	Mg^{2+}-ATPase (µmol Pi/h/mg protein)	Ca^{2+}-ATPase (µmol Pi/h/mg protein)	Na^{+}, K^{+}-ATPase (µmol Pi/h/mg protein)
NC	5.50 ± 0.02[a]	2.84 ± 0.01[a]	3.80 ± 0.06[a]
PC	3.70 ± 0.03	1.50 ± 0.04	2.40 ± 0.03
XP150	5.90 ± 0.03[a]	2.80 ± 0.01[a]	3.50 ± 0.02[a]
XP300	6.10 ± 0.023[a]	3.08 ± 0.03[a]	4.10 ± 0.04[a]
Donep5	4.00 ± 0.01[a]	3.15 ± 0.05[a]	3.90 ± 0.05[a]
Cur 100	4.90 ± 0.02[a]	2.88 ± 0.01[a]	4.15 ± 0.01[a]

NC: Normal Control; PC: Positive Control; Donep: Donepezil; Cur: Curcumin. The values are expressed as mean ± sem. [a]$p < 0.05$ vs positive control (PC).

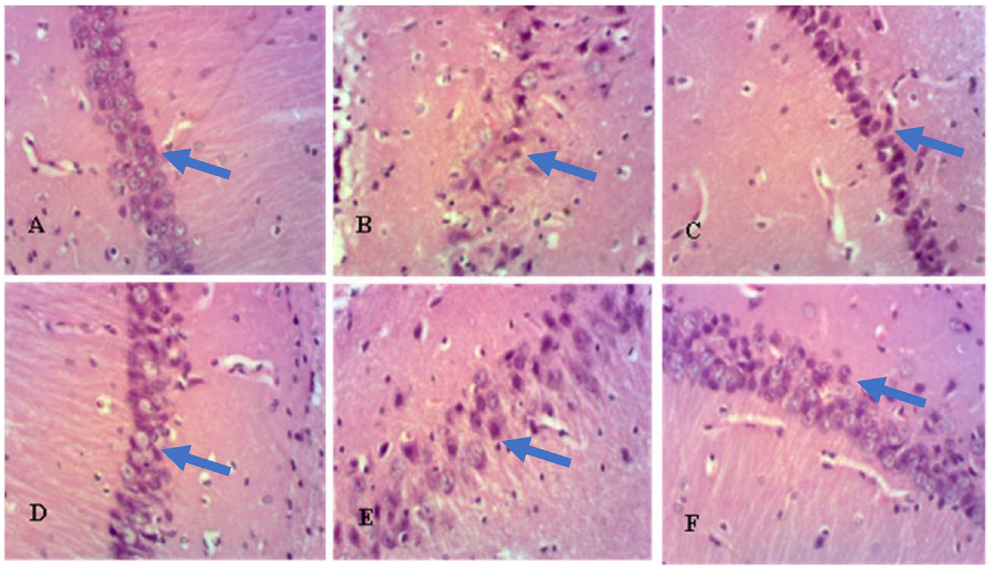

Figure 3. Microphotographs of Ammon's-horns (X400) of the hippocampus; Hematoxylin-eosin staining. Animals were treated without or with $AlCl_3$ (75 mg/kg/BW *per os*), or cotreated with $AlCl_3$ (75 mg/kg/BW *per os*) and extract of *Xylopia parviflora* (150 and 300 mg/kg/BW/day *per os*) or reference compounds (Donepezil, 5 mg/kg/BW/day *per os*) and (Curcumin, 100 mg/kg/BW/day *per os*) for 60 days. A. Normal control; B. Positive control; C. Donepezil; D. Curcumin; E. *Xylopia Parviflora* 150; F. *Xylopia Parviflora* 300.

have properties that facilitate learning and memory after administration during the different phases of the test. For the second behavioural test on locomotion, rats treated with $AlCl_3$ showed a reduction in the number of movements and an increase in stabilisation. Bowdler and collaborators proved that aluminium exposure in animals and humans results in behavioral changes and intellectual impairment [45]. In fact, motor activity of rat is significantly affected by treatment with aluminium chloride. The increase in the number of movements and the decrease in the time spent in the centre of box for the groups of rats treated with *X. parviflora* extract and the reference drugs indicate the increase in locomotion activity like *Carissa edulis* [46]. *X. parviflora* showed significant improvement in all the behavioral parameters in open field, thus suggesting an improvement in aluminium induced behavioral alterations. These results indicated protective or antagonistic effects of our extracts on/against the action of aluminium chloride. This could be explained by the presence of bioactive substances such as polyphenols and alkaloids in our extracts which have the ability to inhibit the effects of $AlCl_3$. This suggests that our spice, through their bioactive compounds, provide neuroprotection by inhibiting the production of free radicals and the accumulation of ROS. Similarly [46, 47, 48], suggest that extract bioactive compounds act by protecting vulnerable neurons, stimulating neural function (cholinergic neurotransmission in brain), improvements in memory and blood circulation as well as promoting neurogenesis.

In this study, markers of impaired cognitive function were identified by assessing brain atrophy, ionic homeostasisby magnesium (Mg^{2+}) and calcium (Ca^{2+}) quantification and cholinergic system (AChE and BChE) activities in brain. Ingestion of $AlCl_3$ by rats induced a decrease in brain mass compared to rats in the normal control group. This may be explained by the loss of some neurons due to the toxicity of $AlCl_3$. $AlCl_3$ showed to be neurotoxin chemical by affecting the biochemical content of brain, histological alternation of cerebral cortex of the brain, disrupting behavioral activities. Administration of *X. parviflora* extract at doses of 150 and 300 mg/kg BW before $AlCl_3$ administration reduced brain atrophy. The mechanisms leading to neuronal loss are not clearly

established, but the hypothesis of apoptosis has been advanced [49]. Hesperitin and quercetin (flavonoids) have been shown to accumulate in mitochondria and inhibit neuronal apoptosis by acting as free radical scavengers or by activating/phosphorylating proteins that are important signals in cell survival pathways [50]. Thus, phenolic and other bioactive compounds contained in the extract of *X. parviflora* [17] could limit neuronal loss, probably through antioxidant and anti-apoptotic activities. In addition, Al induces the expression of pre-apoptotic genes [51].

The minerals evaluated in this study were important ions for the proper functioning of the central nervous system [52]. Their low brain concentrations or abnormal elevation may be associated with an increased risk of neurological disorders [53]. Studies have shown that Mg^{2+} and Ca^{2+} are critical factors in the control of synaptic density or neuronal plasticity and that impaired cognitive function in patients with Alzheimer's disease is mainly caused by synaptic loss [54]. Intoxicated rats ($AlCl_3$) had low levels of Mg^{2+}, but high levels of Ca^{2+} compared to negative control group. This alteration may be due to decrease of brain ATPase activities which are known to modulate Ca^{2+} and Mg^{2+} homeostasis [43]. A high concentration of Ca^{2+} generates oxidative stress by overconcentration of calcium in mitochondria, activation of neuronal nitric oxide synthase (nNOS) which leads to the production of nitric oxide NO- and formation of peroxynitrite ONOO with O_2 (Table 2 and Table 3) [55]. Al^{3+} also degrades calcium (Ca^{2+}) metabolism by negatively interfering with Ca^{2+} signalling pathways, blocking Ca^{2+} channels. And it competes with this cation for small ligands such as phosphates [56]. The decrease in Mg^{2+} and other brain minerals in patients with Alzheimer's disease compared to normal subjects is believed to be due to a defective transport process characterized by an abnormally low incorporation of Mg^{2+} and an abnormally high aluminum into the neurons of these patients [57]. *X. parviflora* extracts have improved ionic homeostasis by significantly decreasing Ca^{2+} and increasing Mg^{2+} level. On cultures of hippocampal neurons, it has been shown that an increase in Mg^{2+} and a decrease in Ca^{2+} in extracellular fluids lead to a permanent increase in neuronal plasticity [58]. This has led to the hypothesis that the decrease in Mg^{2+} levels leads to an increase in the Ca^{2+}/Mg^{2+} ratio in central nervous system tissues with the consequence that aluminum absorption in these tissues increases, promising neurodegeneration processes [57]. Thus, the extract of *X. parviflora* by increasing Mg^{2+} levels and decreasing cerebral Ca^{2+} levels would promote neuronal plasticity and prevent the neurodegeneration process. The effect of the extract is believed to be due to the ability of the flavonoids contained in the extracts to chelate $AlCl_3$ [59]. The mechanism of antioxidant activity of flavonoids like rutin and quercetin (flavonoid content of *X. parviflora*) may involve either scavenging free radicals and/or by chelating metal ions by hydroxyl functional groups. The chelation of metal ions might inhibit the radical generation, which causes tissue damage [59]. Their ability (flavonoids) to chelate with metal ions has resulted in the emergence of a new category of molecules with a broader spectrum of pharmacological activities.

The results obtained show a significant increase in AChE and BChE activities after administration of $AlCl_3$, this result corroborates the studies by [60, 61], describing strong cholinesterase activation in the presence of $AlCl_3$. Exposure to $AlCl_3$ increased cholinesterase activities through an allosteric interaction between aluminum and the peripheral anionic site of the enzyme molecule [62]. The administration of *X. parviflora* combined a filtrate and a reference drug (Donepezil) 45 min after administration of $AlCl_3$ induced a significant reduction in cholinesterase activities in rats. By inhibiting enzymatic digestion, the concentration and duration of action of acetylcholine and buturylcholine in the synaptic cleft is increased, improving the activation of cholinergic receptors and thus cognitive functions [63]. These results indicate the neuroprotective effect of our extracts, which may be due to the presence of bioactive molecules (flavonoids, alkaloids....). Indeed, several studies have shown the high inhibitory activity of alkaloids present in Amaryllidae, such as galanthamine on acetylcholinesterase [64]. In 2013, Eduardo and colleagues showed that alkaloids had one or more nitrogen atoms in their structure that allowed them to interact with the active site of the enzyme (acetylcholinesterase) and inhibit the activity of the competing acetylcholine substrate. $AlCl_3$-induced memory disorders are associated with increased oxidative stress in the brain [65].

Aluminum-induced neurotoxicity may be related to neurodegeneration caused by increased oxidative stress (Table 4) and deterioration of intracellular signal transduction pathways [66]. Al inflicts its toxic influence by creating an intracellular oxidative environment, a situation conducive to major biological complications and diseases. The results of this study showed that chronic exposure to aluminum significantly decreased levels/activities of SOD, CAT, GSH with increased MDA and NO. Similar results were obtained by [47, 56]. Oxidative damage to rat brain during aging and oxidative stress is responsible for the deterioration of cognitive function [67]. Longterm exposure to aluminum oxidation causes stress and alterations in the antioxidant enzymes in the brain. High levels of MDA suggest the involvement of cellular damage induced by free radicals themselves induced by the toxicity of aluminum. Similar result were mentioned by Yuan et al who stated that Al induced significant increase in MDA concentration in hippocampus and frontal cortex of rats administered daily $AlCl_3$ via drinking water [67]. The presence of high concentratons of NO is believed to be due to the fact that aluminum can stimulate NO production by activating inducible nitric oxide synthase [68] and also by disrupting Ca^{2+} ion homeostasis. Administration of *X. parviflora* elevated levels of defensive antioxidant enzymes with concomitant decreases in NO and MDA, indicating a restoration of oxidative stress indices in the rat brain. SOD plays an important role in the detoxification of the superoxide radical to H_2O_2 by glutathione peroxidase at the expense of GSH. The decrease in SOD, CAT activity may have resulted from an oxidative modification of proteins. Administration of *X. parviflora* in aluminum-treated rats significantly prevented the depletion of GSH levels, suggesting that it is effective in preventing oxidative damage associated with aluminum exposure. Therefore, we can propose that the likely elevation of these enzymes could be a mechanism by which *X. parviflora* fights aluminum-induced neurotoxicity [69]. Administration of *X. parviflora* to aluminum-treated rats significantly rebalanced antioxidant parameters to normal values. This positive effect of this extract on the defence against oxidative stress is probably due to the composition of its secondary metabolites, including alkaloids, polyphenols and flavonoids. Studies by [70, 71] showed that flavonoids, because of their antioxidant properties, can reduce the neurotoxicity of β-amyloid, $AlCl_3$ and H_2O_2 probably via an antioxidant mechanism and via the inhibition of oligomerization of β-amyloid. Amin *et al.* [72] suggested that polyphenols act by protecting vulnerable neurons, stimulating neural function by acting on ions such as Ca^{2+} and Mg^{2+} and blood flow, and promoting neurogenesis. The effects of these phenolic compounds on oxidative stress at the peripheral and cerebral level are believed to be due to their ability to directly trap free radicals produced in these tissues or to suppress their production by decreasing the activity of prooxidant enzymes such as NADPH oxidase and myeloperoxidase and increasing that of antioxidant enzymes such as CAT, SOD [73, 74]. The hippocampus was linked to the memory and learning processes of rats, the ensuing degenerative changes observed in the hippocampal synapses could have caused the memory deficits observed in $AlCl_3$ induced rats in the present study [75]. Histological analysis of hippocampi sections shows that the *X. parviflora* extract reversed the decrease in cell density (pyramidal cells) in the Ammon's horns observed in the $AlCl_3$-treated group. The phytochemical analysis of *X. parviflora* shows the presence of some active secondary metabolites responsibles for the activity of plant. In the hydroethanolic extract of the plant 3 polyphenols are isolated: 3-O-caffeoylquinic acid, p-coumaric acid 4-Oglucoside, 5-O-caffeoylshikimic acid. These compound lowering hepatic lipid accumulation [76]. In the fraction of methanolic extract of plant, secondary and tertiary alkaloidal compounds like isoquinoline alkaloids, 10,11-dihydroxy-1,2-dimethoxynoraporphine and parvinine were isolated, along with 39 known alkaloids [77]. The oil of plant contains monoterpenes and sesquiterpenes [78] and possesses notable

chemopreventive, anti-inflammatory potential [79]. Probably, the differents compound present in this plant can exert the protection of brain cells like observing in this study. Therefore, these results suggest that the *X. parviflora* extract could promote neuronal plasticity, prevents cell loss and can able to stop or slow the progression of degenerative disease [80].

5. Conclusion

The above work showed that aluminum neurotoxicity is mediated by oxidative damage, dysregulation of cognitive deficit, and *X. parviflora* has the potential to counter them. High dose of *X. parviflora* extract (300 mg/kg BW) had improved effectively all alterations induced by aluminum. Our data indicated that *X. parviflora* could be beneficial in countering aluminum-induced neurotoxicity at the biochemical and behavioural levels. The combination of antioxidant, anti-cholinesterase potential and improvement of cognitive impairement of *X. parviflora* may have promising compounds to be tested as potential drugs for the treatment of diseases resulting from oxidative stress and cholinergic dysfunction like Alzheimer's disease.

Declarations

Author contribution statement

Ruth Edwige Kemadjou Dibacto: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Bruno Dupon Akamba Ambamba, Jules Vidal Kamga Nanhah: Performed the experiments; Wrote the paper.

Fils Armand Ella, Christine Fernande Biyegue Nyangono, Martin Fonkoua: Analyzed and interpreted the data; Wrote the paper.

Réné Samuel Minka, Judith Laure Ngondi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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