

## Effect of *Austranella congolensis* on Lipid Profile of Rats' Brain with Experimentally Induced Alzheimer's Disease

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### Abstract

Lipids are essential components of the brain. Changes in brain lipid composition affect the physical and functional properties of the neuronal cell membrane and have been implicated in the pathophysiology of Alzheimer disease (AD). We evaluated in this study the effect of hydroethanolic bark extract of *A. Congolensis* on lipid profile of rats' brain with experimentally induced AD. The experimental model consisted of female rats, which received orally for 8 consecutive weeks a single dose of 50 mg/Kg b.w./day of aluminum trichloride (AlCl<sub>3</sub>) (except control group) followed by distilled water (disease control group) or doses of the extract (150 or 300 mg/Kg b.w./day) or vitamin E (100 mg/Kg b.w./day) or galanthamine (2 mg/Kg b.w. /day). Brain cholesterol, phospholipids and plasmalogen levels and fluidity were evaluated. Brain membranes ATPase activities, Ca<sup>2+</sup>, Mg<sup>2+</sup> and glucose levels were also assayed. Significant modifications of brain lipid composition and fluidity were observed in disease control group compared with control. In addition, Na<sup>+</sup>, K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase activities significantly decreased, the level of intracellular Ca<sup>2+</sup> increased, Mg<sup>2+</sup> content decreased and brain glucose level was significantly higher. Standard drugs (vitamin E, galanthamine) showed a negative effect on brain lipid profile. The extract of 150 mg showed significant improvements of brain lipid profile and fluidity. It also indicated improved brain ATPase activities, ions and glucose brain homeostasis. The extract (150 mg/Kg b.w. dose) by maintaining the brain lipid composition may protect neuronal cell membrane and probably preventing the progression of AD.

**Keywords:** Alzheimer disease, *Austranella congolensis*, glucose metabolism, lipids metabolism, Mg<sup>2+</sup>-ATPase and Na<sup>+</sup>, K<sup>+</sup>-ATPase

### 1. Introduction

The brain is the organ with the highest lipid content after fatty tissue (Sastry, 1985). The brain membranes are mainly composed of phospholipids, cholesterol and glycolipids which determine their fluidity, stability and permeability. The maintenance of the composition and the fluidity of the membranes are necessary factors for the proper functioning of the proteins integrated in the membranes, or even the membrane bound enzymes activities (like Na<sup>+</sup>, K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase) or receptors and ion channels (Farooqui & Horrocks, 1985). Changes in the distribution and the content of these lipids lead to a loss of functions associated with the membranes. For instance, the depletion of membrane cholesterol in primary neuron cultures induces the relocation of N-methyl D-Aspartate receptors (NMDAR) out of lipid rafts, thus inhibiting the cytotoxic response. Inversely, an enrichment of cholesterol in the membranes leads to a grouping of lipid rafts promoting the interaction of proteins and signal transduction in the cell (Marquer *et al.*, 2011). In addition, decrease in phosphatidylserine levels in membranes affects acetylcholine receptor (Sunshine & McNamee, 1992), activities of Na<sup>+</sup>, K<sup>+</sup>-ATPase and diacylglycerol kinase (Spector & Yorek, 1985). Such changes in the levels of cholesterol and phospholipids have been observed in the brain and blood of Alzheimer's disease (AD) patients (Goodenowe *et al.*, 2007; Varma *et al.*, 2018; Goodenowe & Senanayake, 2019).

Alzheimer's disease (AD) is a disease affecting the brain, just as coronary disease affects the heart (Alzheimer's Association, 2019). It is mainly characterized by the presence of extracellular aggregation of amyloid-beta (A $\beta$ ) peptide and intracellular neurofibrillary tangles in the brain (Mattson *et al.*, 2004). The amyloid hypothesis proposed by Hardy & Higgins remains the dominant model of the pathogenesis of AD (Selkoe & Hardy, 2016). In this hypothesis, the A $\beta$  peptide that is generated by enzymatic cleavage of amyloid precursor protein (APP: an

integral membrane protein) interact with neuronal cell membranes (Selkoe, 1993). Two types of A $\beta$ -membrane interactions have been found (Wong *et al.*, 2009). A $\beta$  peptide can insert into the cell membrane and form a pore-like structure that increase the permeability of membrane and may trigger cell death signal. The second interaction is the binding of A $\beta$  peptide onto the surface of the membrane that affects the functions or activities of membrane bound enzymes, receptors and ion channels (Wong *et al.*, 2009). Many studies found that membrane lipid environment is a key factor for A $\beta$ -membrane interactions and its toxicity (Arce *et al.*, 2011; Sani *et al.*, 2011). Recently, it has been demonstrated that lipid model mimic healthy and Alzheimer diseased states of the neuronal membrane interact differently with A $\beta$ 1-42 (Drolle, Negoda Hammond, Pavlov & Leonenko, 2017). In fact, in lipid model mimic diseased state of neuronal membrane, the amyloid toxicity was significantly higher than in lipid model mimic healthy state of neuronal membrane (Drolle *et al.*, 2017). Based on these data, it has been proposed that changes in lipid membrane due to aging and AD may trigger amyloid toxicity as new hypothesis for AD. Therefore, Drolle *et al.*, (2017) suggested that maintaining the lipid composition and structure of neural membranes in healthy state may serve as a new preventive strategy against AD.

The drugs approved for the treatment of AD like galanthamine are only symptomatic treatments. In addition, the number of people with dementia continues to increase and would reach 131.5 million by 2050 (Cummings *et al.*, 2017). There is a great need to prevent or effectively treats this disease (Cummings *et al.*, 2017). Bioactive compounds such as terpenoids, flavonoids, sterols, alkaloids extracted from plants are known as important sources of potential agents for treatment or preventing AD (Jesky & Hailong, 2011; Man *et al.*, 2012). The genus *Austranella* or *Mimusops* belongs to the family of Sapotaceae (Jahan *et al.*, 1995), *Mimusops congolensis* (*Austranella congolensis*), one of species of this genus is indigenous to Cameroon (Fokou, 2006). *Mimusops* species are constantly used in Indian traditional medicine. *Mimusops elengi* L. is traditionally used as cardiotoxic or for its hypotensive and antibacterial activity. It has been indicated that, administration of ethanolic bark extract of *M. elengi* L. (100, 300, 600 mg/kg, *p.o*) in Triton WR-1339 induced hyperlipidemia rats, significantly reduce levels of triglyceride and total cholesterol while increasing HDL levels (Ghaisa *et al.*, 2008). Hanumanthacha & Milind (2012), also found that ethanolic bark extract of *M. elengi* (100, 200 mg/kg *p.o*) improve memory in mice. The bark of *A. congolensis* is used traditionally in Cameroon like *M. elengi* L. for its cardiotoxic or hypotensive properties. Previously, phenolic compounds (24-feruloyltetracosanoic acid and (+)-catechin), sterol ((24R)-Stigmast-7,22(E)-dien-3- $\alpha$ -ol (chondrillasterol)) and pentacyclitriterpenoids (taraxerol 3-hexacosanoate, taraxerol 3-tetracosanoate, taraxerol 3-docosanoate, taraxerol and taraxerone) have been found as constituents of the barks of *A. congolensis* (Fokou, 2006). Most of these compounds were also found in the barks of the *Mimusops* species. Additionally, Pentacyclitriterpenoids and plant sterol are compounds able to cross the blood-brain barrier and accumulate in the brain (Pradesa *et al.*, 2011; Vanmierlo *et al.*, 2012). Due to their structural similarity with cholesterol, these compounds are modulators of lipid membrane physical properties (Abboud *et al.*, 2016; Haralampiev *et al.*, 2017), cholesterol-dependent cellular processes and lipid metabolism (Yang *et al.*, 2004). The present study, therefore evaluate the effect of *A. congolensis* on lipid profile of rats' brain with experimentally induced AD.

## 2. Methods

### 2.1 Plant Material and Preparation of Hydroethanolic Extract

The leaves and barks of *A. congolensis* were collected in the East Region of Cameroon. The plant material was identified at the National Herbarium of the Institute of Agricultural Research for Development (IRAD), Yaoundé Cameroon. Plant barks were cut into small pieces of 2-5cm and dried in the laboratory at room temperature. The dried barks were ground to fine powder using a grinder. A sample of 200 mg fine powder was extracted with 1000 mL of ethanol: water (50:50 v/v) extractant for 48 h at room temperature, and then filtered. The obtained filtrate was evaporated and the crude extract was stored until used.

### 2.2 Animals and Experimental Model

#### 2.2.1 Animals

The experiment was performed on female Wistar rats. Body weight was 250-300 g. The rats were housed four in a cage, at a constant room temperature (22  $\pm$  1  $^{\circ}$ C) under a 12 h Light: 12h Dark (light 08.00-20.00 h) cycle and acclimated 1 week before use. Food and water were provided *ad libitum*. Animals were cared for in accordance with the principles of the "Guide to the Care and Use of Experimental Animals" (Committee on Care and Use of Laboratory Animals, 1985).

### 2.2.2 Experimental Protocol

The animals were randomly divided into 6 groups of 8 rats each (table 1). Eight rats were used as control group. The remaining rats received a single dose of aluminiumtrichloride(*p.o*). One hour later, the animals received doses of the extract or vitamin E (alpha tocopherol) or galanthamine and distilled water for control and disease control groups. Doses of aluminum, extract or reference drugs were prepared in distilled water and administered once daily (5 mL/kg b.w.*p.o*) for 8 consecutive weeks. Vitamin E served as standard drug for protecting lipid cell membrane and galanthamine served as standard drug for AD treatment. The weight of animals was monitored weekly with an electronic scale throughout the study.

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
DC	Disease Control	Aluminium (50 mg/Kg bw/day) + Distilled water
AC150	Test group 1	Aluminium (50 mg/Kg bw/day) + hydroethanolic extract of <i>A. congolensis</i> (150 mg/Kg bw/ day)
AC300	Test group 2	Aluminium (50 mg/Kg bw/day) +hydroethanolic extract of <i>A. congolensis</i> (300 mg/Kg bw/ day)
VE100	Standard treatment 1	Aluminium (50 mg/Kg bw/day) + vitamin E (100 mg/Kg bw/ day)
GAL2	Standard treatment 2	Aluminium (50 mg/Kg bw/day) + galanthamine (2 mg/Kg bw/ day)

### 2.3 Brain and Blood Tissue Collection and Preparation

At the end of the experiment period, animals were sacrificed by decapitation after 12 hours of fasting. Blood samples were collected from trunk in tubes containing EDTA and centrifuged to 1500 g at 4 °C for 15 min. The supernatant containing the plasma was collected and stored at -20 °C. The brains, liver, kidneys and heart were rapidly removed, weighed and thoroughly washed with isotonic saline. Each brain was mid-sagittal divided into 2 portions. The first portion (right hemisphere) was homogenized in 10 volume ice-cold (0-4 °C) buffer (50mM TRIS-HCl, pH 7.4 and 300 mM sucrose). Then, the homogenate was centrifuged at 1000 g for 10 minutes to remove nuclei and debris. The resulting supernatant were then immediately stored at -80 °C and used for the enzymes activities assay and for the determination of magnesium, calcium and glucose contents. The second portion of brain (left hemisphere) was used for lipid profile analysis.

### 2.4 Lipid Analysis

#### 2.4.1 Lipid Extraction

Total lipids were extracted according to the method of Folch *et al.*, (1951). Briefly, the tissues were homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (0.5 g in 10 mL of solvent mixture). The homogenates were centrifuged to recover the liquid phase. Then, the solvent was washed with 0.2 volume (4 mL for 20 mL) 0.9% NaCl solution. After vortexing some seconds, the mixture was centrifuged at 2000 g to separate the two phases. The lower chloroform phase containing lipids were stored at -20 C until used.

#### 2.4.2 Determination of Total Phospholipids Content

Total phospholipids was determined according to the method of Stewart (1980), based on the formation of a colored complex between phospholipids and ammonium ferrothiocyanate. An aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 2 mL of chloroform. One millimeter of reagent [aqueous solution of iron trichloride (27 g/L) and ammonium thiocyanate (30 g/L)] was added and the mixture homogenized in a tube shaker (Vortex) for 30 seconds, then centrifuged for 10 minutes at 6000 rpm. The absorbance of the lower chloroform phase was measured at 488 nm against the blank (2 mL chloroform + 1 mL of reagent). A standard range was carried out for quantities varying from 10 to 100 µg of phosphatidylcholine (PC). The phospholipids content were expressed as µg equivalent phosphatidylcholine (µg Eq PC) per mg of total protein (µg Eq. PC/mg protein).

#### 2.4.3 Determination of Plasmalogens Content

Plasmalogen content was determined according to the method of Gottfried and Rapport (1962), based on the reaction of vinyl ethers content of plasmalogen with iodine. An aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 0.5 mL of methanol. Then 0.5 mL of iodine reagent (6 x 10<sup>-4</sup> N iodine in 3 % aqueous KI) was added. The mixture was

stirred vigorously for 1 minute and left at room temperature for 10 minutes. After addition of 4.0 mL of 95% ethanol, the absorbance was read at 355 nm against a sample blank in which 0.5 mL of 3% KI was substituted for the iodine reagent. The molar extinction coefficient of iodine was reported to 27,500. The plasmalogen content was expressed in brain as nmoles of vinyl groups/mg total protein and in erythrocytes and plasma expressed as mmoles of vinyl groups/L (mM).

#### 2.4.4 Determination of Cholesterol and Triglycerides Levels

##### **Cholesterol**

Cholesterol levels were determined enzymatically according to the method of Richmond (1973), following a kit protocol (Chronolab references number 101-0576; 101-0593; 101-0526, 101-0440). Briefly, an aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 0.5 mL of methanol. 10  $\mu$ L of this methanolic extract was taken in a glass test tube and 1 mL of working reagent was added. The solutions were mixed and incubated for 10 minutes at room temperature (25  $^{\circ}$ C), and the absorbance of the brain sample or plasma sample (10  $\mu$ L plasma + 1 mL working reagent) or standard (10  $\mu$ L standard cholesterol + 1 mL working reagent) were read against the blank at 505nm. The cholesterol content were expressed in brain as  $\mu$ g/mg total protein and expressed in plasma as mmol/L (mM).

##### **Triglycerides**

Triglycerides levels were also determined enzymatically according to the method of Fossati and Principe (1982), following a kit protocol (Chronolab reference numbers 101-0241; 101-0016; 101-0268; 101-0052 and 101-0053). Briefly, 1mL of working reagent was added to 10  $\mu$ L of plasma sample or standard. The solutions were mixed and incubated for 10 minutes at room temperature (25  $^{\circ}$ C), and the absorbance of the samples and standard were read against the blank at 505 nm. Triglycerides content were expressed as mmol/L (mM).

#### 2.5 Enzymes Activities, Calcium, Magnesium and Glucose Levels Assay

##### 2.5.1 ATPases Activities Assay

$\text{Na}^+$ ,  $\text{K}^+$ -ATPase and  $\text{Mg}^{2+}$ -ATPases activities were assayed by a method adapted from Rohn *et al.*, (1993). The method is based on determining the amount of phosphate released by enzymatic cleavage of ATP in absence or presence of ouabain. For each rat brain sample, two test tubes were used. 450  $\mu$ L of assay buffer (NaCl 150 mM, KCl 15 mM, MgCl 15 mM EGTA 0.1 mM in histidineHCl-TRIS 30 mM pH 7.5) containing 50  $\mu$ g of brain homogenate protein was added to the first tube. The second tube was made in same condition however, ouabain was added in the final concentration of 1.5 mM. The tubes were pre-incubated at 37  $^{\circ}$ C for 10 minutes, and the assay was started with the addition of 50  $\mu$ L of ATP. $\text{Na}_2$ -TRIS (final concentration 5 mM) thus, the final volume of reaction was 500  $\mu$ L. After 60 min, the reaction was terminated by the addition of 500  $\mu$ L of 15% (w/v) of coldtrichloroacetic acid solution. The level of inorganic phosphate present in solution was quantified following the calorimetric method of (Baginski *et al.*, 1967) and was used as a measure of ATPase activity. The absorbance values obtained were converted to activity values by linear regression using a standard curve of sodium monobasic phosphate that was included in the assay procedure. The  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity was determined by subtracting the ouabain sensitive activity ( $\text{Mg}^{2+}$ -ATPase activity) from the total ATPase activity level (first tube). The activity of ATPases was expressed as  $\mu$ mol of inorganic phosphate liberated from ATP by 1 mg of protein for a duration of one hour ( $\mu$ mol Pi/mg protein/hour).

##### 2.5.2 Calcium, Magnesium and Glucose Levels

##### **Calcium**

Calcium ions ( $\text{Ca}^{2+}$ ) were measured following the kit Sigma-Aldrich protocol (Number MAK022) and expressed as  $\mu$ mol/mg total protein.

##### **Magnesium**

Magnesium ions ( $\text{Mg}^{2+}$ ) were measured following the kit Sigma-Aldrich protocol (Number MAK026) and expressed as  $\mu$ mol/mg total protein.

##### **Glucose**

Glucose levels in brain and plasma were measured following the kit protocol Sigma-Aldrich (Number GAGO20) and expressed in brain as  $\mu$ mol/g tissue and in plasma expressed as millimole/L (mM)

#### 2.6 Total Protein

The total protein levels were determined by the method described by Lowry *et al.*, (1951).

## 2.7 Statistical Analysis

The statistical package for social sciences (SPSS) software version 20.0 (Chicago-IllinoisInc.) was used and One-way analysis of variance (ANOVA) with Tukey's test was performed to compare variability amongst the groups. Significant differences were detected at 95% confidence interval and the results obtained were expressed as Mean  $\pm$  Standard Deviation.

## 3. Results

### 3.1 Effect of Extract on Body Weight Variation and Relative Weights of Brain, Liver and Heart

Table 2 shows the initial, final and body weight variation as well as the relative weights of brain, liver and heart. We observed a significant decrease in body weight and brain relative weight in disease control rat in comparison with control rats. Rats treated with extract at 150 mg/Kg b.w. showed a significant lower body weight and brain relative weight loss than disease control group. The vitamin E treated group also showed a significant lower brain relative weight loss compared to disease control group. No significant difference in liver and heart relative weight were observed between groups.

Table 2. Body weight (BW) variation and relative weights of brain, liver and heart

Groups	Initial BW (g)	Final BW (g)	Brain relative weight (g $\times 10^{-3}$ )	Liver relative weight (g $\times 10^{-3}$ )	Heart relative weight (g $\times 10^{-3}$ )
NC	274.4 $\pm$ 19.3	281.6 $\pm$ 13.1 (+2.3%) <sup>a</sup>	7.6 $\pm$ 0.4 <sup>a</sup>	31.5 $\pm$ 2.5 <sup>a</sup>	3.2 $\pm$ 0.2 <sup>a</sup>
DC	279.4 $\pm$ 13.1	264.4 $\pm$ 11.4 (-7.5%) <sup>b</sup>	6.6 $\pm$ 0.2 <sup>b</sup>	28.1 $\pm$ 1.0 <sup>a</sup>	2.9 $\pm$ 0.19 <sup>a</sup>
AC150	274.7 $\pm$ 15.5	274.2 $\pm$ 11.8 (-0.0%) <sup>a</sup>	7.5 $\pm$ 0.33 <sup>a</sup>	26.7 $\pm$ 1.7 <sup>a</sup>	3.1 $\pm$ 0.11 <sup>a</sup>
AC300	276.0 $\pm$ 15.5	266.5 $\pm$ 10.8 (-4.1%) <sup>ab</sup>	6.7 $\pm$ 0.3 <sup>b</sup>	25.0 $\pm$ 1.8 <sup>a</sup>	3.3 $\pm$ 0.12 <sup>a</sup>
VE100	270.0 $\pm$ 9.5	269.25 $\pm$ 9.2 (-0.6%) <sup>ab</sup>	7.4 $\pm$ 0.0 <sup>a</sup>	39.0 $\pm$ 1.5 <sup>a</sup>	3.4 $\pm$ 0.10 <sup>a</sup>
GAL2	279.0 $\pm$ 15.3	278.0 $\pm$ 13.0 (-0.3%) <sup>a</sup>	6.7 $\pm$ 0.2 <sup>b</sup>	30.0 $\pm$ 0.6 <sup>a</sup>	3.1 $\pm$ 0.16 <sup>a</sup>

NC: Normal Control rats; DC: Disease Control rats; AC<sub>150</sub>: 150 mg /kg BW of the extract; AC<sub>300</sub>: 300 mg /kg BW of the extract; VE<sub>100</sub>: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean  $\pm$ Standard deviation, values with different superscripts down the column are statistically different p < 0.05.

### 3.2 Effect of the Extract on Brain and Blood Lipid Composition Changes

#### 3.2.1 Effect of the Extract on Brain Lipid Composition

Table 3 reveals brain lipid parameters of the rats. In disease control rat, the total cholesterol (CHL) increased significantly (+115.8%), and the total phospholipids (PHL) content were not significantly modified compared with control rats. Also, the cholesterol to phospholipid (CHL/PHL), molar ratio significantly increased (+93.6%) and the plasmalogen content significantly decreased (-35.7%) compared with control rat. In the group treated with the extract (150 mg/Kg b.w), the level of total cholesterol and the CHL/PHL molar ratio were significantly lower than those of disease control rats and statistically comparable to those of control. Plasmalogen levels were significantly higher in the group treated with the extract (150 mg/Kg b.w) than in disease control group and also statistically comparable to those of control group. The alteration of brain lipid profile in groups treated with vitamin E or galanthamine was higher than in disease control group.

Table 3. Brain lipid parameters of rats

Groups	Cholesterol ( $\mu$ g/mg protein)	Phospholipids ( $\mu$ g/mg protein)	Cholesterol/phospholipids molar ratio (mol :mol)	Plasmalogens (nM/mg protein)
NC	193.6 $\pm$ 13.8 <sup>a</sup>	525.0 $\pm$ 12.3 <sup>a</sup>	0.8 $\pm$ 0.0 <sup>a</sup>	158.8 $\pm$ 4.9 <sup>a</sup>
DC	417.9 $\pm$ 22.1 <sup>b</sup>	529.5 $\pm$ 10.0 <sup>a</sup>	1.6 $\pm$ 0.0 <sup>b</sup>	101.9 $\pm$ 4.0 <sup>b</sup>
AC150	215.3 $\pm$ 7.8 <sup>a</sup>	726.9 $\pm$ 10.9 <sup>b</sup>	0.6 $\pm$ 0.0 <sup>a</sup>	179.5 $\pm$ 7.8 <sup>a</sup>
AC300	461.9 $\pm$ 15.9 <sup>b</sup>	656.0 $\pm$ 13.5 <sup>ab</sup>	1.3 $\pm$ 0.0 <sup>b</sup>	143.1 $\pm$ 2.7 <sup>ab</sup>
VE100	424.6 $\pm$ 9.4 <sup>b</sup>	392.9 $\pm$ 21.5 <sup>c</sup>	2.03 $\pm$ 0.1 <sup>b</sup>	94.80 $\pm$ 3.3 <sup>b</sup>
GAL2	395.3 $\pm$ 6.8 <sup>b</sup>	269.8 $\pm$ 6.7 <sup>c</sup>	2.1 $\pm$ 0.0 <sup>b</sup>	103.8 $\pm$ 2.4 <sup>b</sup>

NC: Normal Control rats; DC: Disease Control rats; AC<sub>150</sub>: 150 mg /kg BW of the extract; AC<sub>300</sub>: 300 mg /kg BW of the extract; VE<sub>100</sub>: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean  $\pm$ Standard deviation, values with different superscripts down the column are statistically

different  $p < 0.05$ .

### 3.2.2 Effect of the Extract on Blood Lipid Composition

Table 4 indicates the blood lipid parameters of the rats. In disease control rats, there were significant increases in plasma triglyceride (+104.8%) and plasma cholesterol (+54.1%) levels compared to control rats. The plasmalogens content in plasma and erythrocytes were not significantly modified between these two groups. Rats treated with the extract (150 mg/Kg b.w) showed lower cholesterol and triglycerides levels than those of disease control group and statistically similar to those observed in control group. Vitamin E treated-rats also showed decrease in plasma cholesterol and triglycerides but lower than those observed in rats treated with the extract (150 mg/Kg b.w).

Tableau 4. Blood lipid parameters of rats

Groups	Plasma triglycerides (mM)	Plasma cholesterol (mM)	Plasma plasmalogens (mM)	Erythrocytes plasmalogens (mM)
NC	0.41±0.003 <sup>a</sup>	0.85±0.034 <sup>a</sup>	0.32±0.004 <sup>a</sup>	0.19±0.001 <sup>a</sup>
DC	0.84±0.001 <sup>b</sup>	1.31±0.029 <sup>b</sup>	0.32±0.025 <sup>a</sup>	0.18±0.005 <sup>a</sup>
AC150	0.32±0.002 <sup>a</sup>	0.84±0.036 <sup>a</sup>	0.30±0.007 <sup>a</sup>	0.22±0.002 <sup>a</sup>
AC300	0.65±0.011 <sup>b</sup>	1.52±0.031 <sup>b</sup>	0.33±0.005 <sup>a</sup>	0.21±0.001 <sup>a</sup>
VE100	0.48±0.004 <sup>a</sup>	1.09±0.014 <sup>b</sup>	0.31±0.006 <sup>a</sup>	0.21±0.001 <sup>a</sup>
GAL2	0.86±0.013 <sup>b</sup>	1.12±0.034 <sup>b</sup>	0.30±0.001 <sup>a</sup>	0.20±0.002 <sup>a</sup>

NC: Normal Control rats; DC: Disease Control rats; AC<sub>150</sub>: 150 mg /kg BW of the extract; AC<sub>300</sub>: 300 mg /kg BW of the extract; VE<sub>100</sub>: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different  $p < 0.05$ .

### 3.3 Effect of the Extract on Brain ATPases Activities and Associated Cations Contents

The results indicated a significant decreases in Na<sup>+</sup>, K<sup>+</sup>-ATPase (-32%) and Mg<sup>2+</sup>-ATPase (-26,5%) activities, and depletion in Mg<sup>2+</sup> contents in disease control rats compared to normal control rats (Table 5). The rats treated with the extract (150 and 300 mg/Kg b.w) showed a significant increase in Na<sup>+</sup>, K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase activities and concomitant increases in Mg<sup>2+</sup> levels and decrease in Ca<sup>2+</sup> levels. Vitamin E and galanthamine treatments also significantly increased Na<sup>+</sup>, K<sup>+</sup>-ATPase activity and increased the Mg<sup>2+</sup> and decreased Ca<sup>2+</sup> contents compared with disease control.

Table 5. Na<sup>+</sup>, K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase activities, and Mg<sup>2+</sup> and Ca<sup>2+</sup> levels in brain

Groups	Na <sup>+</sup> , K <sup>+</sup> -ATPase (μmol Pi/h/mg protein)	Mg <sup>2+</sup> -ATPase (μmol Pi/h/mg protein)	Mg <sup>2+</sup> (μmol/mg protein)	Ca <sup>2+</sup> (μmol/mg protein)
NC	2.2±0.02 <sup>a</sup>	4.0±0.01 <sup>a</sup>	30.2±3.7 <sup>a</sup>	15.9±1.0 <sup>ab</sup>
DC	1.4±0.03 <sup>b</sup>	2.2±0.01 <sup>b</sup>	20.5±2.2 <sup>b</sup>	18.4±1.3 <sup>b</sup>
AC150	2.1±0.03 <sup>a</sup>	5.3±0.01 <sup>a</sup>	28.3±2.3 <sup>a</sup>	13.8±1.3 <sup>a</sup>
AC300	2.7±0.04 <sup>a</sup>	4.8±0.02 <sup>a</sup>	34.8±2.2 <sup>a</sup>	11.6±2.1 <sup>a</sup>
VE100	3.0±0.06 <sup>a</sup>	3.2±0.01 <sup>a</sup>	33.6±3.9 <sup>a</sup>	12.4±1.3 <sup>a</sup>
GAL2	2.6±0.10 <sup>a</sup>	2.7±0.01 <sup>ab</sup>	31.4±3.7 <sup>a</sup>	10.5±1.7 <sup>a</sup>

NC: Normal Control rats; DC: Disease Control rats; AC<sub>150</sub>: 150 mg /kg BW of the extract; AC<sub>300</sub>: 300 mg /kg BW of the extract; VE<sub>100</sub>: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different  $p < 0.05$ .

### 3.4 Effect of the Extract on Brain and Plasma Glucose Levels

The findings revealed plasma and brain glucose contents in rats (Table 6). In disease control rats, the level of glucose in brain was significantly increased, whereas no significant change was observed in plasma glucose level compared to control rats. Rats treated with the extract at both doses (150 and 300 mg) showed significant low levels in cerebral glucose content as compared with disease control rats. Brain glucose level in rats treated with

the extract at 150 mg/Kg b.w was significantly lower than in control group. Treatments with reference drugs also lowered the brain glucose level compared with disease control group.

Table 6. Brain and plasma glucose levels

Groups	Brain glucose ( $\mu\text{mol/g}$ tissus)	Plasma glucose (mM)
NC	2.1 $\pm$ 0.1 <sup>a</sup>	5.3 $\pm$ 0.2 <sup>a</sup>
DC	10.6 $\pm$ 0.3 <sup>b</sup>	5.9 $\pm$ 0.3 <sup>a</sup>
AC150	1.3 $\pm$ 0.0 <sup>c</sup>	5.7 $\pm$ 0.2 <sup>a</sup>
AC300	3.0 $\pm$ 0.1 <sup>d</sup>	6.14 $\pm$ 0.0 <sup>a</sup>
VE100	5.2 $\pm$ 0.1 <sup>d</sup>	7.0 $\pm$ 0.2 <sup>a</sup>
GAL2	4.8 $\pm$ 0.1 <sup>d</sup>	5.6 $\pm$ 0.3 <sup>a</sup>

NC: Normal Control rats; DC: Disease Control rats; AC<sub>150</sub>: 150 mg /kg BW of the extract; AC<sub>300</sub>: 300 mg /kg BW of the extract; VE<sub>100</sub>: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean  $\pm$ Standard deviation, values with different superscripts down the column are statistically different  $p < 0.05$ .

#### 4. Discussion

The analyze of lipid profile of rats in this study, both at the brain and peripheral levels revealed a significant change in lipid composition in disease control rats compared to normal control. The molar ratio of CHL/TPL an accepted index of membrane fluidity (Senault *et al.*, 1990), also increased in disease control rats, indicating decreased fluidity (Senault *et al.*, 1990). These results corroborates with several studies showing the capacity of Al<sup>3+</sup> to induce changes in lipid composition and the physical properties of plasma membrane. Indeed, Pandya, Dave & Katyare (2003), examined the myelin lipid profile of rats after exposure to AlCl<sub>3</sub>. They observed significant increase in cholesterol (CHL) content with a decrease in the total phospholipid (TPL) content and in the TPL / CHL molar ratio. More so, changes in phospholipids composition of the myelin membrane were similar to phospholipid profiles reported in AD brains. Sarin *et al.*, (1997) studied the lipid composition and various membrane-bound enzymes in different regions of monkey brain following chronic aluminum exposure indicating similar observations. In human neuroblastoma cells, Verstraeten *et al.*, (2002) found that, Al<sup>3+</sup> (10 to 100  $\mu\text{M}$ ) caused a significant loss of membrane fluidity, and increased the relative content of lipids in gel phase while promoted lipid rearrangement through lateral phase separation.

On the other hand, we found that the brain and blood lipid profiles of rats receiving extract particularly at the dose of 150 mg/Kg were statistically similar to those of control. This therefore highlights a strong effect of the extract at this dose on protecting lipid membranes or on modulating lipid metabolism. Such effects may be due to the presence of pentacyclitriterpenes (taraxerol 3-hexacosanoate, taraxerol 3-tetracosanoate, taraxerol 3-docosanoate, taraxerol and taraxerone) or sterol ((24R)-Stigmast-7,22(E)-dien-3- $\alpha$ -ol (chondrillasterol)) previously isolated from this plant by Fokou (2006). In fact, it has been shown that, taraxerol is able to incorporate itself in DPPC (Dipalmitoyl-phosphatidylcholine) bilayers and is more potent than cholesterol to decrease the DPPC gel phase (Rodriguez *et al.*, 1997). In addition, spinasterol [(22 E, 24 S)-5 $\alpha$ -stigmasta-7, 22-dien-3 $\beta$ -ol] a stereo isomer of chondrillasterol showed the ability to partially take over the role of natural cholesterol on the structural level of lipid membrane (Haralampiev *et al.*, 2017). Thus, it is possible that by incorporating themselves into the brain lipid membrane, taraxerol and chondrillasterol probably in our extract displace cholesterol and therefore modified its homeostasis or metabolism. It is well known that plant pentacyclitriterpenoids or sterol affect critical regulatory pathways of lipid metabolism. In fact, the metabolism of cholesterol in cell is finely regulated by transcription factors like SREBP (Sterol Regulatory-Element Binding Proteins), by low density lipoprotein (LDL) receptors, or by ATP-binding cassette (ABC) transporters receptors (Shimano & Sato, 2017). In BV2 microglial cells, Kharrassi *et al.*, (2014) found that spinasterol can modulate the gene expression of two nuclear receptors, the Liver X receptors (LXR - $\alpha$  and LXR- $\beta$ ) by targeting ABCA1 and ABCG1 genes. Yang *et al.*, (2004) discovered that stigmasterol, inhibits the treatment of SREBP-2 and reduces the synthesis of cholesterol. Pentacyclitriterpenes have been indicated to inhibit the expression of the ACAT gene (Acyl-coenzyme A: cholesterol acyltransferase) which controls ACAT, a key enzyme that take part in the metabolism of cholesterol and plasma fatty acids (Liu *et al.*, 2007). All of these may be likely biochemical mechanisms by which the compounds present in our extract have lowered the cholesterol and triglyceride contents in the brain and plasma. In addition, plasmalogen constitute a particular class of membrane glycerophospholipids (GP) having a unique structural characteristic (a vinyl ether group, -O-CH = CH-, at the sn-1 position ofglycerol backbone instead of the usual ester function). In neurons, the plasmalogens are enriched

in polyunsaturated fatty acids (PUFAs), decosahexonoic acid and arachidonic are the most abundant (Braverman & Moser, 2012). The presence of these PUFAs in plasmalogens may render them very susceptible to lipid peroxidation and oxidative damage. Therefore the high contents of total phospholipids and plasmalogen observed in the groups having received the extract could be due to the protective effect of the extract against the oxidation of the PUFAs contained in these phospholipids. It should also be noted that the vitamin E and galanthamine reference drugs used in this study enhanced brain lipid alteration. Such effect of vitamin E was also observed by other authors. In fact, Subudhi *et al.*, (2009), observed that vitamin E supplementation (200 mg/kg b.w of vitamin E orally for 30 days) in hypothyroid rats altered plasma lipid profile by increasing the levels of plasma total cholesterol, non-HDL cholesterol and decreasing those of HDL-cholesterol. Vitamin E supplementation also increased lipid peroxidation and protein carbonylation. It is therefore important that, additional studies be done to better understand the effects of vitamin E supplementation or galanthamine administration in brain lipid profile in the context of AD.

The activity of membrane proteins such as Na<sup>+</sup>, K<sup>+</sup>-ATPase depends strongly on the surrounding lipid environment. Enzymes evaluation showed a significant reduction in the activities of Na<sup>+</sup>,K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase in disease control rats compared with normal control. Decrease in Na<sup>+</sup>, K<sup>+</sup>-ATPase activity has been shown as an early marker of AD (Yu *et al.*, 2016). The complete hydrolytic activity of the enzyme depends on an association between the phospholipids and the cholesterol of the plasmatic membrane (Habeck *et al.*, 2015). This might explain the lower Na<sup>+</sup>, K<sup>+</sup>-ATPase activity of disease control rats, accounted for by their low phospholipids levels and high cholesterol level compared with control rats or with rats treated with the extract. In addition, we found in disease control group an alteration of ions (Ca<sup>2+</sup> and Mg<sup>2+</sup>) homeostasis. This alteration may be due to decrease of brain ATPase activities which are known to modulate Ca<sup>2+</sup> and Mg<sup>2+</sup> homeostasis (Sanui *et al.*, 2003). Mg<sup>2+</sup> content can modulate the activity of Mg<sup>2+</sup> kinase-dependent enzymes such as glycolysis kinase (hexokinase, phosphofructokinase and pyruvate-kinase) and therefore take part in the glucose homeostasis (Cohn & Roth, 1983). Glycolysis is also regulated during Na and K movements by the activity of phosphofructokinase (Erecinska & Dagoni, 1990). All of this suggest a relationship between the activities of Mg<sup>2+</sup>-ATPase, Na<sup>+</sup>,K<sup>+</sup>-ATPase and glycolysis or glucose homeostasis. We found in this study that, brain-glucose level in disease control rats was significantly elevated than in control group. Therefore, diminished cerebral ATPase activities probably account for elevated brain-glucose observed in these rats by impairing intracellular glucose utilization through glycolysis. Another potential explanation for elevated brain-glucose could be that of impaired glucose transporter (GLUT) activity, resulting to decrease brain-glucose uptake and consequent cerebral 'hypometabolism'. These results are in agreement with An *et al.*, (2018) who found that higher brain glucose levels in AD were related to lower rates of glycolysis and lower glucose transporter 3 (GLUT3). In the rats treated with the extract, we observed a low level of glucose in brain suggesting a high rate of glucose utilization with probably high glycolytic rate and high energy-production.

## 5. Conclusion

We evaluated in this study the effect of hydroethanolic extract of *A. congolensis* on lipid profile of rats' brain with experimentally induced Alzheimer's disease. Our results revealed the ability of this extract to maintain brain lipid composition and fluidity at healthy state. The activities of membrane-bound ATPases, Ca<sup>2+</sup> and Mg<sup>2+</sup> homeostasis, and glucose metabolism in brain were also improved by this extract more than reference drugs. It was concluded that the extract may protect neuronal cellular membranes against Aβ peptide interaction and toxicity, thereby preventing the progression of AD. However, we should chemically characterize this extract and show the underlying mechanisms, by which extract compounds interfere with regulatory pathways of lipid metabolism. Furthermore, the high dose of extract (300 mg/Kg b.w) had not improved brain lipid alteration. Further studies on dose-effect of extract will be carried out to better understand this result.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

NNDJ carried out the study and wrote the manuscript; NJL contributed to conception, design and analysis of data, and OJE assisted with and supervised the manuscript writing. All authors have read and approved the final manuscript.

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