

Full Length Research Paper

Neuroprotective effect of *Khaya grandifoliola* against aluminium chloride (AlCl₃) induced neurotoxicity in rats

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Aluminium chloride (AlCl₃) is known as a potent environmental neurotoxin causing progressive neurodegeneration in the brain. *Khaya grandifoliola* (KG) is used in Cameroon in traditional medicine for its therapeutical properties. Thus, the aim of this study was to evaluate the neuroprotective effect of *K. grandifoliola* against AlCl₃-induced neurotoxicity in rats. The KG25-fraction was obtained by maceration of the *K. grandifoliola* bark powder followed by fractionation by flash chromatography. The neuronal dysfunction induced by AlCl₃ was evaluated on 36 rats divided into 6 groups of 6 rats each during 45 days. On day 46 after 24 h of fasting, the rats were sacrificed. AST, ALT, PAL, catalase activity, glutathione level, peroxidized lipid level and acetylcholinesterase activity were assessed. AlCl₃-induced anxiety was reduced by administration of the *K. grandifoliola* 25-fraction. The oxidative stress response of the *K. grandifoliola* 25 fraction showed a significant increase (p<0.05) in catalase activity at the dose of 225 mg/kg body weight, glutathione and lipid peroxidation levels followed the same pattern. Acetylcholinesterase activity showed a significant increase in group III (positive control) and a significant decrease (p<0.05) in the experimental groups. The *K. grandifoliola* 25 fraction effectively protects the brain of rats against the toxic effects of AlCl₃ at doses (75 and 225 mg/kg) body weight, respectively.

Key words: *K. grandifoliola* 25, AlCl₃, anxiety, neurotoxicity, oxidative stress.

INTRODUCTION

Aluminium (Al) is the most abundant metal found in the environment, leading to constant human exposure through various sources such as drinking water, cooking utensils, fruits, certain drugs, and deodorants (Taus et al., 2013). Due to the fact that aluminium weakens and

easily crosses the blood-brain barrier, it acts on the central nervous system (CNS) as a neurotoxin, with its concentration being higher in certain sensitive regions of the brain such as the hippocampus and the cortex (Thangarajan et al., 2013; Prakash and Kumar, 2013).

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Several biochemical, epidemiological, and neuropathological studies have suggested aluminium's involvement in the pathogenesis of certain neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease (AD) (Kaumar and Gill, 2014). According to *in vitro* and *in vivo* studies, the neurotoxicity of aluminium is more implicated in AD; it induces cognitive deficits followed by other characteristics of AD, allowing it to be used to induce a model of AD (Prakash and Kumar, 2013).

AD is a neurodegenerative disease characterized by a progressive and irreversible deterioration of cognitive faculties and also characterized by two types of lesions: neuritic plaques (extracellular) and neurofibrillary tangles or tau deformation (intraneuronal) (Wang et al., 2016). Approximately 24 million people worldwide suffer from dementia, 60% of which are due to AD (Ferri et al., 2005). A study conducted at the Bastos clinic in Yaoundé, Cameroon, showed that over 6 years, 20.15% of patients admitted to the clinic were diagnosed with neurological disorders including AD, Parkinson's disease, and various other dementias (Teugueu et al., 2013). For the treatment of this disease, cholinesterase inhibitors are the first line of prescription drugs available for patients with mild to moderate forms of AD.

By inhibiting the hydrolysis of acetylcholine at synapses, these drugs restore the level of neurotransmitters in affected neurons in patients. In the symptomatic treatment of moderate to severe forms, memantine has been approved. Another possibility is the use of anti-inflammatory and antioxidant therapies. However, some of these drugs, in addition to being expensive, are associated with many undesirable effects as well as a loss of effectiveness over time.

With these limitations and the increase in the elderly population, AD represents a relevant health problem in the world, hence the need to seek new, more effective and less expensive treatments that can protect the brain against damage. In order to search for new molecules or mixtures of active compounds, scientists continue to investigate the biological activity of medicinal plants. For instance, *Khaya grandifoliola* (KG) is a plant used in traditional medicine in Cameroon and West Africa in the treatment of various liver conditions (Moundipa et al., 2002). Previous work done on this plant has shown that it is used for the treatment of liver-related diseases, malaria, arthritis, anemia, and fever (Njyou et al., 2016; Olowokudejo et al., 2008). Pharmacological investigations have shown antimalarial, antibacterial, anti-inflammatory, and antifungal properties (Bickii et al., 2000; Mukaila et al., 2021). Additionally, it also has hepatoprotective (Olowokudejo et al., 2008; Galani et al., 2016) and antioxidant (Galani, 2009; Njyou et al., 2013) properties and contains polyphenols and flavonoids as the main bioactive compounds, as well as an acetylcholinesterase inhibition property (Amougou, 2012).

Recent studies conducted on the hydroethanolic extract of *K. grandifoliola* have shown a preventive effect of this plant against scopolamine-induced cognitive impairment through the inhibition of acetylcholinesterase activity, as well as the regulation of certain oxidative stress parameters (Fils et al., 2022).

Also, work carried out on the active fraction *K. grandifoliola* 25 has revealed that this fraction, purified from *K. grandifoliola* crude extract, has anti-inflammatory activities on N9 microglia by inhibiting the NO and pro-inflammatory cytokines induced by LPS *in vitro* (Brice et al., 2022). From these observations, and with the aim of exploring new possible activities of this plant, we hypothesized that *K. grandifoliola* has neuroprotective activity. Thus, the present study sought to examine the neuroprotective capacity of the *K. grandifoliola* 25 fraction obtained from the crude extract of the barks of *K. grandifoliola* to protect the brains of rats against the toxicity induced by aluminium chloride.

METHODOLOGY

Plant material

The barks of *K. grandifoliola* were harvested in June 2015 in Foumban (West/Cameroon) and identified in the national herbarium C.DC (52661 YA).

Experimental animals

Albino rats of the Wistar strain, male and female, aged 8 to 12 weeks and weighing between 150 and 250 g were used. These rats were bred in the animal facility of the Laboratory of Pharmacology and Toxicology of the University of Yaoundé I.

Reagents and equipment

The list of equipment used is given in appendix (I); the reagents used were supplied by the Sigma (Hamburg, Germany) and PROLABO (Paris, France) laboratories.

Preparation of the crude extract with a 1:1 v/v methylene chloride/methanol mixture of *K. grandifoliola*

The bark was cut, dried at room temperature and ground. The preparation of crude extract from the powder obtained was carried out according to the steps presented in Figure 1. The crude extract obtained was weighed and the extraction yield was calculated by the following formula:

$$\text{Yield} = \text{mass of extract} \times 100 / \text{mass of powder}$$

Preparation of the *K. grandifoliola* 25 fraction from the crude plant extract

The active fraction *K. grandifoliola* 25 (25% methylene chloride/

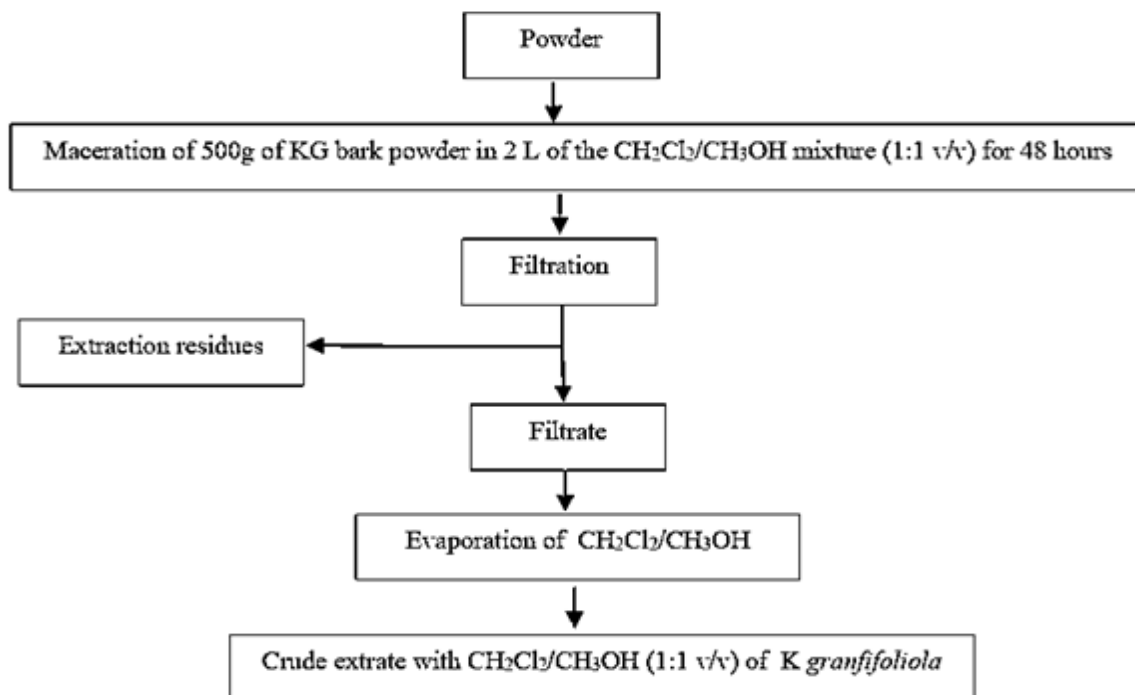


Figure 1: steps for preparing Khaya grandifoliola raw extract

methanol fraction of *K. grandifoliola*) was prepared by "flash chromatography." With its high yield, volatile nature, and ability to dissolve many organic compounds, methylene chloride is an ideal solvent for many chemical processes and is reputed to be the least toxic of the simple chlorohydrocarbons. The crude extract of *K. grandifoliola* (68.92 g) was dissolved in methanol inside a flask. Then, an equivalent mass of silica powder was added, and the mixture was thoroughly mixed by adding a small amount of methanol to ensure homogeneity. This mixture was concentrated using a rotary evaporator. The resulting paste was dried, crushed, and fractionated by flash chromatography. For this chromatography, several solvents or solvent mixtures were used in the following order: methylene chloride, methylene chloride/ methanol (95:5 v/v), methylene chloride/methanol (90:10 v/v), methylene chloride/methanol (75:25 v/v), and methanol. The solvent change was made when the filtrate appeared clear. The residues obtained constituting the fractions were weighed, kept in clean bottles, and the yields calculated using the formula:

$$\text{Yield} = \text{mass of the fraction obtained} \times 100 / \text{mass of the crude extract}$$

Study of the neuroprotective properties of the *K. grandifoliola* fraction

Neuronal dysfunction in rats was induced using the aluminium chloride-induced neurotoxicity model. For this, 6 groups of 6 rats each, including 3 males and 3 females, were formed and treated as follows: Group I: negative control (carboxymethylcellulose 1% (CMC 1%)), Group II: extract control (225 mg/kg of KG25), Group III: positive control (150 mg/kg of aluminium chloride (AlCl₃)), Group IV: experimental (150 mg/kg of AlCl₃ + 25 mg/kg of KG25), Group V: experimental (150 mg/kg of AlCl₃ + 75 mg/kg of KG25), Group

VI: experimental (150 mg/kg of AlCl₃ + 225 mg/kg of KG25). The rats were weighed daily and force-fed using a gastroesophageal tube. During the first 30 days of treatment, animals in groups I and II received distilled water while those in groups III, IV, V, and VI were given aluminium chloride. From day 31 to day 45, animals in group I received CMC (1%), animals in groups III, IV, V, and VI stopped receiving AlCl₃ and those in groups II, IV, V, and VI were treated with the *K. grandifoliola* 25 fraction at the respective doses of 25, 75, and 225 mg/kg of body weight. On days 14, 30, and 45, the animals were subjected to behavioral tests to assess their level of anxiety (behavioral dysfunction). This test was performed between 7:00 and 11:00 a.m., and the dysfunction was assessed using the elevated plus maze assay. On day 46, the rats were sacrificed after fasting for 12 h with access to ad libitum water. After sacrifice by decapitation, blood was collected for serum preparation by centrifugation (3000 rpm, 4°C, 15 min). The serum was used to assess the level of hepatotoxicity markers, namely ALT, AST, ALP; and in parallel, the effect of *K. grandifoliola* 25 on other markers. The recovered brain was used for the preparation of homogenates.

Tests to assess cognitive dysfunction

Raised cross maze

Cognitive dysfunction was assessed using the elevated plus maze test according to the method proposed by (Pellows et al., 1985). The apparatus is made up of four arms (two closed and two open) each measuring 50 cm long and 10 cm wide, and facing each other. The closed and open arms are connected via the central area (5 × 5 cm) to form the (+) sign. Two of the arms, named "closed arms" (denoted CA), and opposite, are cloistered by three 40 cm high walls. The other two arms, called "open arms" (denoted OA), also



Figure 2. Evaluation of biochemical parameters from serum and brain homogenates: blood collection and serum preparation.

opposite, are completely open. The labyrinth is located at a height of 50 cm above the ground. Two hours after the treatment, the rat was placed in the central platform of the maze, in front of the open arm. The number of entries as well as the time spent in each type of arm was recorded for 5 min.

After each trial, all the arms and the central air of the maze were cleaned with ethanol (70°) to eliminate any odors, in order to prevent any influence due to the olfactory sense. The percentage of time spent in the open arms of the maze is calculated by the formula:

$$\% \text{TSOA} = \text{TSOA} \times 100 / (\text{TSOA} + \text{TSCA})$$

where TSOA= time spent in open arm, TSCA= time spent in closed arm.

On the 46th day of the experiment (24 h after administration of the last dose of the drug) all the animals were sacrificed by decapitation and the blood collected in dry tubes then centrifuged at 3000 rpm for 15 min at 4°C. Serum was collected and stored in 1.5 ml Eppendorf tubes at -20°C. Figure 2 shows the evaluation of biochemical parameters from serum and brain homogenates: blood collection and serum preparation.

Preparation of brain homogenates

After dissection of the skulls, the brain was quickly excised and washed with a saline solution (NaCl 0.9%), weighed and stored at -20°C. Later, the brains were homogenized using a mortar placed on ice and 10% homogenate was prepared using 50 mM Tris-HCl pH 7.4 containing 300 mM sucrose, then centrifuged later at 3000 rpm/min for 30 min at 4°C to harvest the supernatant. The supernatant of each sample was aliquoted into 1.5 ml Eppendorf tubes for estimation of acetylcholinesterase activity and other antioxidant parameters (LPO peroxidized lipids, GSH-reduced glutathione, catalase CAT).

Evaluation of biochemical parameters in serum

Kinetic determination of serum transaminases

The methodology used was proposed by Wróblewski and Ladue (1956)

Glutamate pyruvate transaminase/Alanine aminotransferase (GPT/ALT)

In a tank, 500 µL of reagent 1, 500 µL of reagent 2, and 100 µL of serum were introduced. The mixture was then homogenized and the OD red after 1 min at 340 nm at times 0, 1, 2, 3 successively using a spectrophotometer. The amount of ALT was calculated using the formula below. $\text{GPT/ALT U/L} = \Delta A/\text{min} \cdot 1750$ Glutamate oxaloacetate transaminase (GOT/AST). In a tank we introduced 500 µL of reagent 1, 500 µL of reagent 2 and 100 µL of serum then homogenized and read the OD after 1 min at 340 nm at times 0, 1, 2, 3 successively using a spectrophotometer. The amount of ALAT was calculated using the formula below $\text{GOT/AST U/L} = \Delta A/\text{min} \cdot 1750$. Kinetic assay of Alkaline Phosphatase (ALP) In a tank we introduced 1 ml of reagent 1.4 ml of reagent 2 and 10 µL of serum then homogenize and read the OD after 1 min at 340 nm at times 0, 1, 2, 3 successively using a spectrophotometer. The amount of ALT was calculated using the formula:

$$\Delta A/\text{min} \times 1190 = \text{U/L of PAL.}$$

Evaluation of biochemical parameters in brain homogenate

Protein assays

In a test tube, 50 µL of brain homogenate and 0.950 µL of distilled water were introduced. In another tube (blank), was introduced 1000µL of distilled water. To each of these tubes was added 4 ml of

the biuret reagent respectively. After shaking the tubes, the colour was allowed to develop at room temperature for 20 min. And, the optical density was read at 540 nm against blank. The calibration range was established from a standard solution of bovine serum albumin (BSA).

Determination of lipid peroxidation

0.1 ml of brain homogenate supernatant was introduced in dry tubes. In another tube called blank was introduced 0.1 ml of tris/HCl-sucrose buffer. Then into each of these tubes was introduced 0.1 ml of 8.1% dodecyl sulfate (SDS), followed by the addition of 1 ml of 30% acetic acid (pH 3.5) and 1.5 ml of thiobarbituric acid and the volume was reduced to 2.8 ml with distilled water. The resulting mixture was homogenized, sealed with glass beads and heated in a water bath for 1 h at 95°C. Then 1 ml of distilled water was added, followed by the addition of 2.5 ml of n-butanol. The mixture obtained was centrifuged at 3000 rpm for 10 min at 4°C, then the supernatant collected and the optical density of the pink coloration developed was read at 532 nm against the blank. The MDA concentrations were calculated by the formula (Ohkawa et al., 1979):

$$[\text{MDA}] (\mu\text{M/g}) = \frac{\text{OD} \times V_{\text{ts}} \times 10^6}{\epsilon_{\text{MDA}} \times V_{\text{sp}} \times Q_{\text{p}}}$$

where OD = optical density, V_{ts} = total volume of supernatant (μl), MDA = molar extinction coefficient of MDA = $1.56 \times 10^5 \text{ m}^{-1} \cdot \text{cm}^{-1}$, V_{sp} = volume of supernatant sampled for the assay (μl), Q_{p} : amount of protein in the total volume of the supernatant in g, l = optical path (1 cm).

Assessment of catalase activity According to the method proposed by Claiborne (1985)

One milliliter of phosphate buffer (0.1 M pH 7.2) and 975 μl of freshly prepared H_2O_2 (0.091 M) were introduced into the blank and test spectrophotometric tanks, then 25 μl of brain homogenate in the tests. The absorbance of the mixture obtained was read at 560 nm for 2 min and recorded every minute against the blank and the enzyme activity was calculated according to the formula:

$$\text{Activity (IU/min/mg protein)} = (2.3033/\Delta T) \times (\log A_1/A_2) / Q_{\text{prote}}$$

where A_1 : Absorbance at the first minute, A_2 : Absorbance at the second minute, ΔT : Time interval in minutes, and Q_{prote} : quantity of protein in the flight of supernatant used (mg).

Assay of cellular glutathione

Twenty microliters of brain homogenate and 20 μl of buffer were introduced respectively into the test tubes and blank, then 3 ml of Ellman's reagent (0.05 mM) DTNB in buffer phosphate (0.1 M pH 6.5). After homogenization, the tubes were incubated at room temperature for 1 h, then the absorbance was read at 412 nm against the blank and the amount of glutathione cell was calculated by the formula:

$$[\text{Glutathione}] (\text{mM/mg protein}) = \frac{\text{OD} \times V_{\text{ts}} \times 10^3}{\epsilon_{\text{glutathione}} \times V_{\text{sp}} \times l \times Q_{\text{p}}}$$

where OD = optical density, V_{ts} = total volume of the supernatant (μl), glutathione = molar extinction coefficient of glutathione = $13,600 \text{ m}^{-1} \cdot \text{cm}^{-1}$, V_{sp} = volume of supernatant sampled for the assay (μl); Q_{protein} = total amount of protein in the supernatant (mg); l = optical path (1 cm).

Assessment of acetylcholinesterase activity

50 μL of brain homogenate was introduced into a cuvette containing 950 μL of buffer Tris-HCl (50 mM pH 8); then 100 μL of dithiobis nitrobenzoic acid was added to the tank followed by the addition of 20 μL of acetylthiocholine iodide (ATCI). Thiocholine reacts with DTNB to produce a yellow color which was read on a spectrophotometer at 412 nm. Enzyme activity was calculated using the formula:

$$\text{Specific activity } (\mu \text{ mole of hydrolyzed acetyl choline iodide})/\text{min/mg of proteins} = (\Delta A/\text{min}) \times 50 \mu\text{L}/Q_{\text{p}}$$

Statistical analysis

The experiments were conducted in duplicate, and the results obtained were presented as means \pm standard deviations. Statistical analysis was performed using GraphPad Prism software version 5.0 for Windows. Comparisons of means between the treated groups and the control and intoxicated groups were carried out using one-way analysis of variance (ANOVA). For groups showing significant differences in variances, means were compared using the Bonferroni test. A probability threshold of $p < 0.05$ was considered statistically significant.

RESULTS

Extraction and fractionation yields

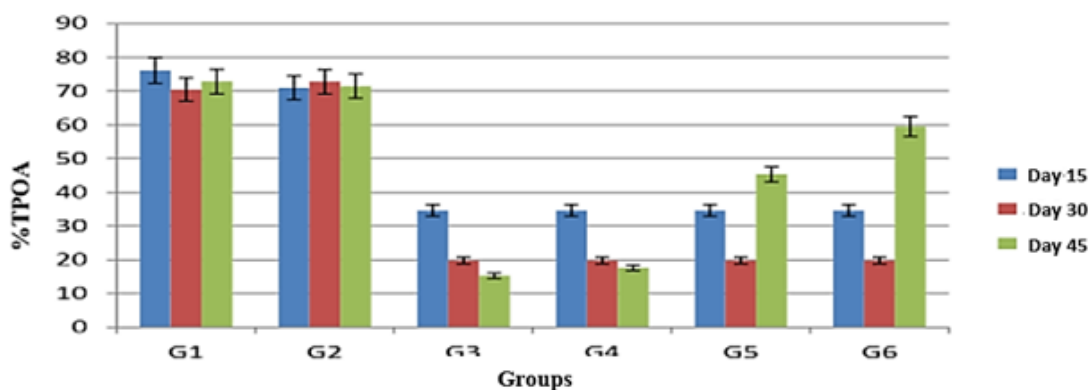
The different solvent systems used for the preparation of the extract allowed us to obtain the crude extract (RE) and the different fractions: Fc (methylene chloride fraction); F5 (5% methylene chloride/methanol fraction (95/5, v/v)); F10 (10% methylene chloride/methanol fraction (90/10, v/v)); F25 (25% methylene chloride/methanol fraction (75/25, v/v)); FMeOH (Methanol fraction) with yields shown in Table 1.

Effect of *K. grandifoliola* 25 on behavioral parameters in aluminium chloride-induced neurotoxicity

Figure 3 illustrates the effect of *K. grandifoliola* 25 on the anxiety levels of rats in the different groups, assessed using the elevated plus maze. The results are depicted as the percentage of time spent in the open arms of the maze. It is evident that animals in groups 1 and 2, which respectively received 1% CMC (carboxymethyl cellulose) and only the *K. grandifoliola* 25 fraction (225 mg/kg of body weight), exhibited more time spent in the open arms of the maze, indicating reduced anxiety. Conversely, in group 3, where animals received only AlCl_3 for 30 days, a decrease in the percentage (34.644% for day 14 and

Table 1. Extraction and fractionation yield of the plant's crude extract.

| Yields (%) | |
|-------------------------|----------------------------|
| Raw extract or fraction | <i>Khaya grandifoliola</i> |
| RE | 13.78 |
| F _C | 2.27 |
| F ₅ | 4.37 |
| F ₁₀ | 7.50 |
| F ₂₅ | 12.00 |
| FM _{eOH} | 25.23 |

**Figure 3.** Effect of KG25 fraction on animal anxiety level. G (1, 2, 3, 4, 5 and 6) = Groups.

19.712% for day 30) of time spent in the open arms of the maze was observed. This trend was also observed in animals of groups 4, 5, and 6 until day 30. However, from day 31 to day 45, animals treated with respective doses of 25, 75, and 225 mg/kg of body weight showed an increase in the percentage (17.57, 45.365, and 59.504% for day 45) of time spent in the open arms of the maze, indicating a decrease in anxiety levels in these rats.

Effect of *K. grandifoliola* on the activity of some serum parameters in aluminium chloride-induced neurotoxicity

Effect of *K. grandifoliola* fraction on the activity of serum transaminases

Figure 3a and b, respectively shows the effects of the active fraction of our plant on the activities of ALAT and ASAT enzymes in the sera of rats in the different groups. The results indicate that the administration of aluminium chloride at a dose of 150 mg/kg of body weight does not lead to a significant increase in the activity of transaminases ALAT and ASAT in the serum of poisoned

and treated animals compared to those receiving only carboxymethyl cellulose and to those intoxicated and untreated.

The effect of the *K. grandifoliola* 25 on the activity of alkaline phosphatase (Figure 4) illustrates the impact of the *K. grandifoliola* 25 fraction on the activity of alkaline phosphatase PAL in the sera of rats in the different groups (Figure 5). The results obtained demonstrate that the administration of aluminium chloride at a dose of 150 mg/kg of body weight does not lead to a significant increase in the activity of alkaline phosphatase in the serum of poisoned and treated rats compared to rats receiving only carboxymethyl cellulose and to those intoxicated and untreated.

Effect of *K. grandifoliola* 25 on total protein concentration in rat brain

Figure 6 illustrates the effect of the *K. grandifoliola* 25 fraction on the total protein concentration in the brain homogenate of rats in the different groups. The results indicate that the administration of aluminium chloride at a

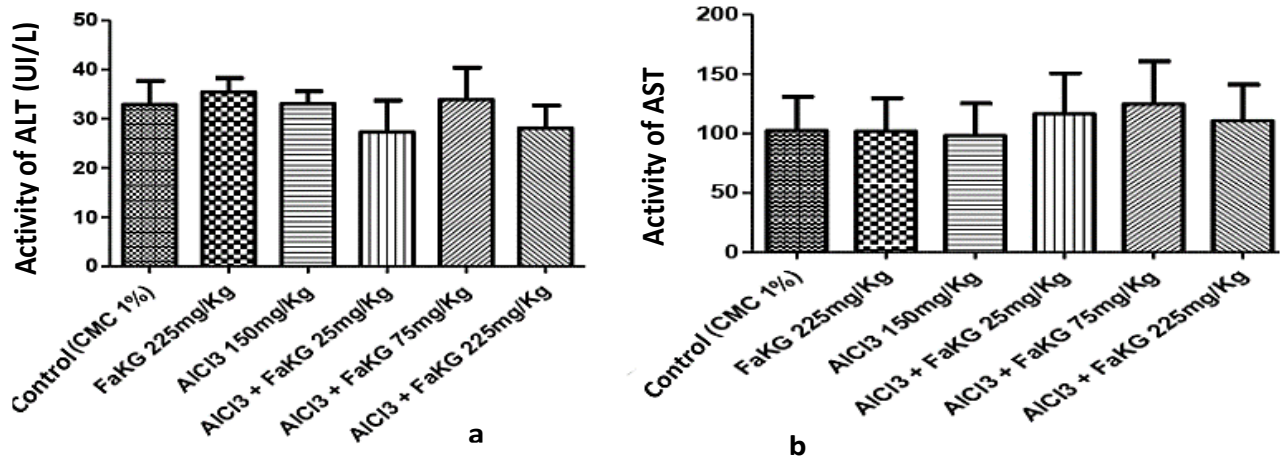


Figure 4. Effect of the KG25 Fraction on the activities of ALT (a) and AST (b) in serum in IU/L.

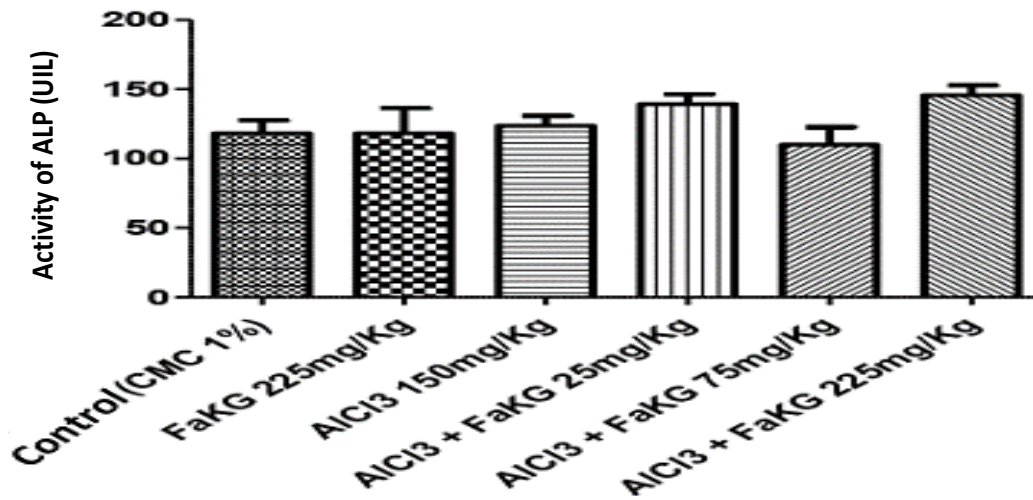


Figure 5. Effect of KG25 Fraction on ALP activity in serum.

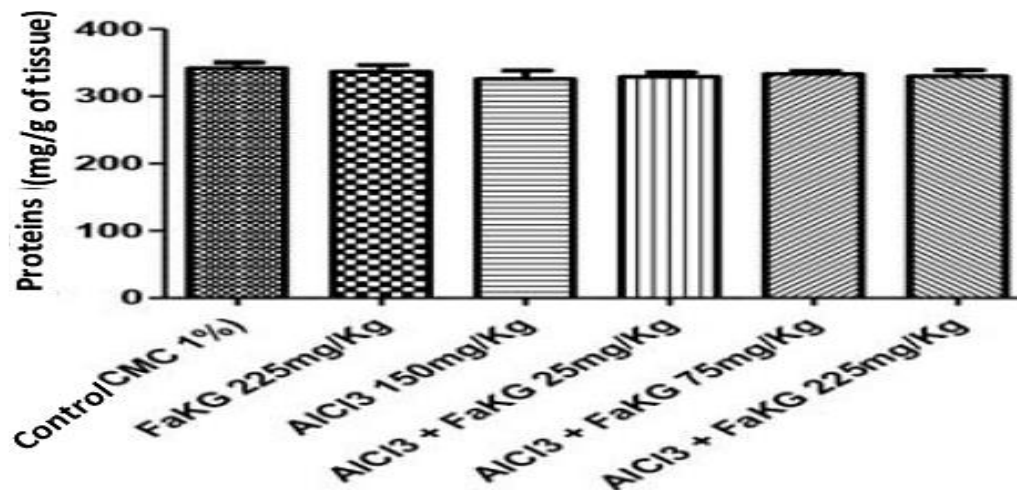


Figure 6. Effect of KG25 fractions on total protein concentration in brain homogenate.

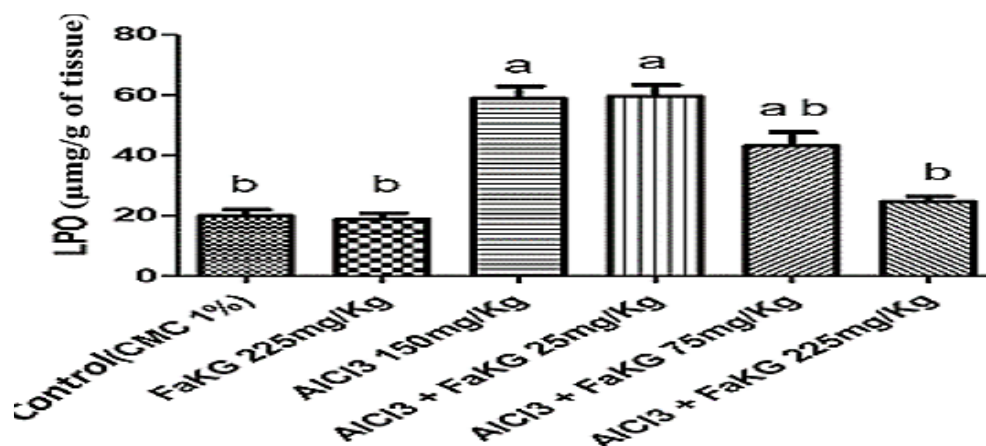


Figure 7. Effect of treatment with the KG25 fractions on the peroxidation of membrane lipids in the brain of rats poisoned with aluminium chloride.

dose of 150 mg/kg of body weight did not lead to a significant modification of the protein concentration in the brain homogenate of poisoned and treated animals compared to those receiving only carboxymethyl cellulose and to those intoxicated and untreated.

Effect of *K. grandifoliola* 25 fractions on some parameters of oxidative stress and on acetylcholinesterase activity in aluminium chloride-induced neurotoxicity

***Effect of K. grandifoliola* 25 on lipid peroxidation**

Figure 7 shows the effect of treatment with the *K. grandifoliola* 25 fractions at different concentrations on the peroxidation of membrane lipids in the brains of rats poisoned with aluminium chloride. The intoxication of rats with AlCl₃ caused a very significant increase ($p < 0.0001$) in the production of peroxidized lipids (LPO). The induction of lipid peroxidation by aluminium chloride was significantly attenuated by the administration of *K. grandifoliola* 25 fractions ($p < 0.01$).

***Effect of K. grandifoliola* 25 fractions on catalase activity and cellular glutathione level**

Figures 8 and 9 show the effect of the active fraction *K. grandifoliola* 25 on the activity of catalase and on the level of cellular glutathione. Analysis of the results shows that poisoning of rats with aluminium chloride induces a significant drop ($p < 0.001$) in catalase activity and cellular glutathione levels. On the other hand, treatment with our *K. grandifoliola* 25 fraction significantly increases catalase activity and cellular glutathione levels compared

to the intoxicated and untreated group.

***Effect of K. grandifoliola* 25 on acetylcholinesterase activity**

Figure 10 shows the effect of treatment with the active moiety at different concentrations on acetylcholinesterase activity in the brains of rats poisoned with aluminium chloride. Acetylcholinesterase is a key enzyme in cholinergic neurotransmission and is a marker of loss of cholinergic neurons in the brain. The intoxication of rats with aluminium chloride caused a very significant increase ($p < 0.0001$) in the activity of this enzyme. On the other hand, the administration of our active fraction significantly attenuated the activity of the enzyme compared to the group intoxicated with AlCl₃ and not treated.

DISCUSSION

Aluminium is considered as an environmental factor that contributes to neurodegenerative disorders; with the tolerable daily intake being between 3 and 12 mg/day. However, repeated and habitual exposure to aluminium can cause neurotoxicity (Dey and Singh, 2022). In this study, we aimed at evaluating the neuroprotective effect of *K. grandifoliola* against aluminium chloride-induced neurotoxicity in rats.

In the present study, rats that were treated with aluminium chloride showed behavioral alteration and increased anxiety as shown by the elevated plus-maze test compared to normal groups that did not receive the toxicant (groups 1 and 2) and experimental groups (groups 4.5 and 6). The results obtained showed that the

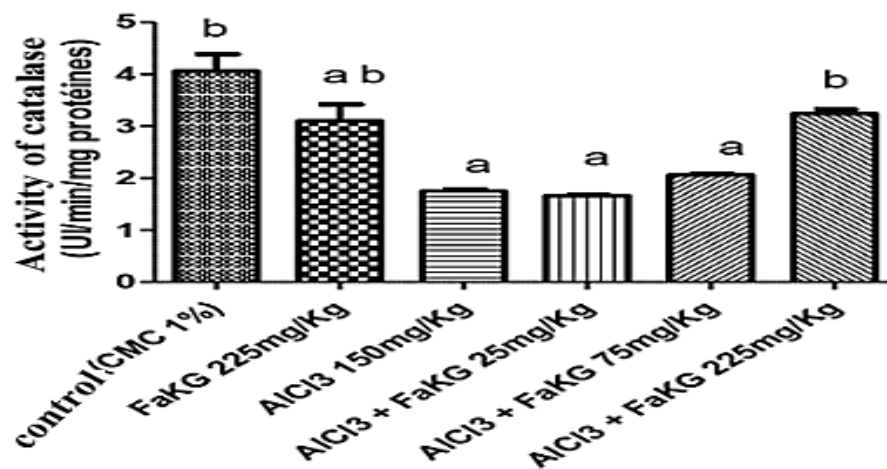


Figure 8. Effect of KG25 fractions on catalase activity of chloride aluminium poisoned rats.

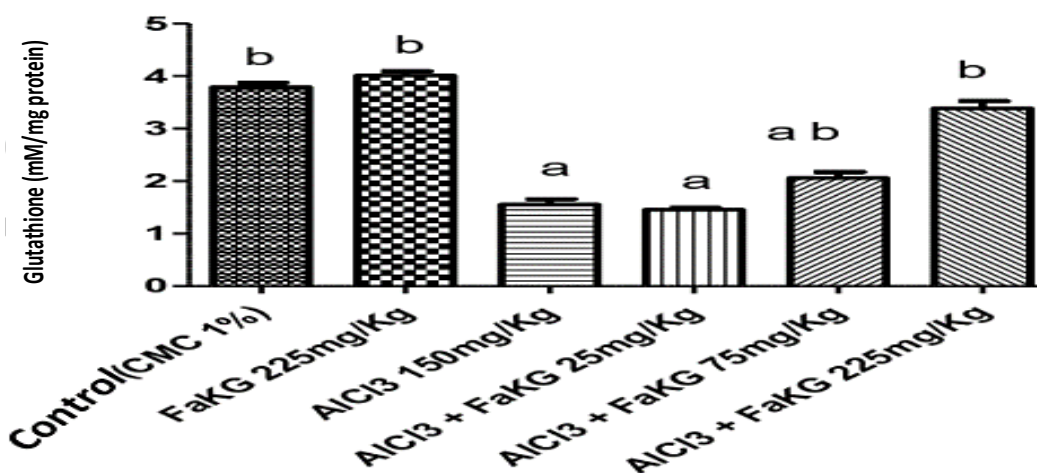


Figure 9. Effect of KG25 fractions on the cellular glutathione level of rats poisoned with aluminium chloride.

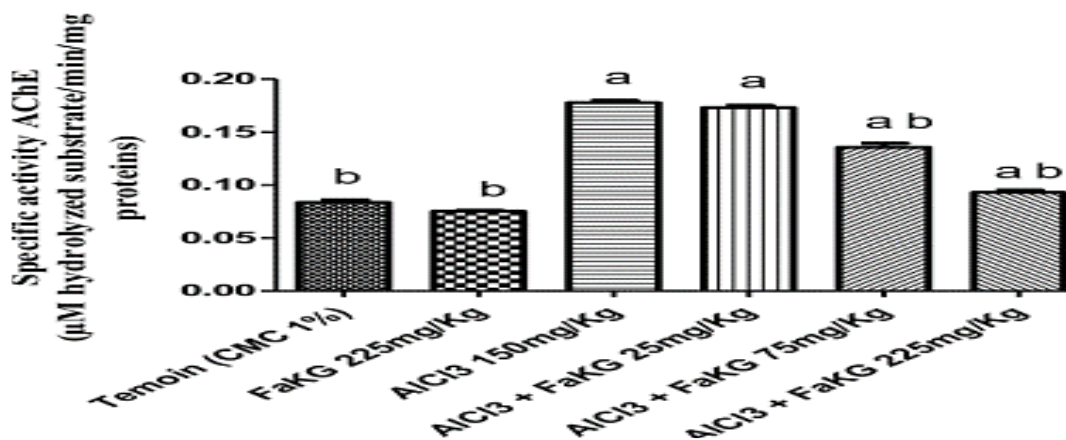


Figure 10. Effect of treatment with the KG25 fractions on the activity of acetylcholinesterase in the brain of rats poisoned with aluminum chloride.

oral administration of aluminum chloride caused an increase in anxiety as observed in the cross maze in rats (Gentsch et al., 1987). This phenomenon can be attributed to the ability of aluminum chloride to interfere with downstream effector molecules such as: cyclic GMP involved in long-term potentiation (Lakshmi et al., 2015). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are generally considered markers of liver damage.

Aluminium chloride was found to be involved in liver damage, especially observed during chronic exposure or administration (Adebayo et al., 2012). In this study, aluminum chloride was administered subchronically. The results of ALT, AST and PAL activities showed no significant difference in the intoxicated and treated groups compared to the CMC control group and the untreated intoxicated group. This could be explained by the fact that the animals were only exposed to the toxic substance subchronically; also the dose administered in this study was one third of the dose that was established to cause liver damage (475 mg/kg) as demonstrated by Adebayo et al. (2012).

Because oxidative damage was mediated by free radicals, it is necessary to investigate the status of an antioxidant enzyme and compound respectively catalase and glutathione which were involved in the first line of defense against free radical damage under oxidative stress (Amjad and Umesalma, 2015). In previous studies, aluminum had been recognized as pro-oxidant causing peroxidized lipids (lipid peroxidation) in different regions of the brain (cortex and hippocampus) (Chia-Yi et al., 2012). Similarly, aluminium caused alterations in iron homeostasis resulting in excessive free iron ions which will undergo the Fenton reaction and cause oxidative damage ultimately leading to neurodegeneration (Lakshmi et al., 2015). These reasons could explain the decrease in the antioxidant enzyme catalase and the reduced compound glutathione and also the increase in the level of peroxidized lipids in the groups of rats where aluminum chloride was administered compared to the other groups (Chia-Yi et al., 2012). Similarly, the administration of *K. grandifoliola* 25 fractions at different doses improved catalase activity, glutathione level and also attenuated the rise in peroxidized lipids and thus protected animals against oxidative stress induced by aluminum chloride.

Neurodegeneration in different areas of the brain resulted from the deterioration of cholinergic transmission which can occur through two pathways. Firstly, in patients with AD, this may be due to: (i) a decrease in released acetylcholine (ACh) or (ii) a decrease in choline acetyltransferase activity leading to a shortage of acetylcholine (Amjad and Umesalma, 2015).

Secondly, an increase in the enzyme acetylcholinesterase (ACh E) which further accentuates the shortage of acetylcholine at the level of the synapse

by the degradation of the available acetylcholine. This degradation of acetylcholine is abolished by acetylcholinesterase inhibitors and had so far been shown to be effective in the treatment of AD, and in improving cholinergic transmission (Costa et al., 2011; Sung et al., 2019; Mahalakshmi et al., 2015).

Recent studies have suggested that the administration of aluminum increases acetylcholine esterase in the brains of rats (Talita et al., 2016). We also showed that the subchronic administration of aluminium significantly increased the activity of acetylcholine esterase, an effect attenuated by the administration of the *K. grandifoliola* 25 fraction.

The effectiveness of the *K. grandifoliola* 25 fraction could be attributed to its chemical composition. This fraction has been shown to be rich in polyphenols and flavonoids (Amougou, 2012) and also known to have antioxidant and anti-inflammatory properties (Galani, 2009; Njayou et al., 2013); also the anti-acetylcholine esterase property of this *K. grandifoliola* 25 fraction had been demonstrated in vitro (Amougou, 2012). Indeed, the analysis of the composition of *K. grandifoliola* 25 by HPLC identified three main compounds, compounds which have an affinity of bonds for Inos and for MAPK by interacting well with the residues of the active site (Brice et al., 2022). But, to say that the action of the *K. grandifoliola* 25 fraction on cholinergic neurons could have a beneficial effect for AD has yet to be established.

Unfortunately, the mechanism by which *K. grandifoliola* 25 regulates acetylcholine esterase remains unknown. We speculate that it is associated with oxidative damage to neurons because of the pronounced antioxidant effect of *K. grandifoliola* 25. The results presented in this study focus on behavior, antioxidant activity and acetylcholinesterase activity in the brain of rats. The elevated plus maze test showed increased anxiety in rats poisoned with aluminum chloride. Chronic exposure to aluminum would increase the concentration of aluminum in the hippocampus and cerebral cortex of rats (Sung et al., 2019). The results observed in our study are correlated with the parameters of oxidative stress.

The parameters analyzed in the different groups are consistent with no alteration in the liver, either by aluminum chloride or by the active fraction *K. grandifoliola* 25. However, *K. grandifoliola* 25 demonstrated a significant improvement ($p < 0.05$) in activities and enzyme levels at the dose of 225 mg/kg in many aspects of our work (behavioral, antioxidant, and on the activity of acetylcholinesterase). These results are similar to those obtained by Amjad and Umesalma (2015) who showed the protective effect of *Centella asiatica* against aluminum-induced neurotoxicity in different brain regions and to those of Mahalakshmi et al. (2015) and Sung et al. (2019) who showed the antioxidant role of *Clitoria ternatea* extract against aluminum-induced oxidative stress in the hippocampus of albino rats. These

results are also similar to those obtained by Ella et al. (2022) who showed the protective effect of hydroethanolic extract of *K. grandifoliola* against cognitive disorders in brain functions by activating cholinergic and antioxidant systems in rats (Fils et al., 2022).

These effects could potentially correlate with the antioxidant properties observed *in vitro* and *in vivo* (Njayou et al., 2013) as well as with the anti-acetylcholinesterase property observed *in vitro* (Amougou, 2012) with *K. grandifoliola* 25. However, future cellular studies are needed to understand the effect as well as the mechanism of the *K. grandifoliola* 25 moiety on oxidative stress in different experimental systems.

Conclusion

In summary, exposure to aluminum chloride caused neuronal inflammation and induced impairment in memory tasked; it also altered the blood brain barrier and causes changes in cholinergic and noradrenergic neurotransmission resulting in an increase in the generation of free radicals thus causing severe neurotoxicity. The results of this study show that the KG25 fractions effectively protected the brain of rats against the toxic effects of aluminum chloride at doses (75 and 225 mg/kg, respectively).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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