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Effects of *Kalanchoe pinnatum* Leaf Extract on Biochemical and Behavioral Parameters of Aluminum Chloride Induced Hippocampal Toxicity on Wistar Rats

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Abstract
Aluminum chloride ($AlCl_3$) is known to induce hippocampal toxicity, leading to oxidative stress, neuro-inflammation, and cognitive deficits, mimicking conditions such as Alzheimer's disease. This study evaluates the effects of *Kalanchoe pinnatum* leaf extracts on the biochemical and behavioral parameters in Wistar rats exposed to $AlCl_3$ induced neurotoxicity. Male Wistar rats were randomly assigned to four groups: control, $AlCl_3$-treated, and $AlCl_3$-treated groups supplemented with low and high doses of *Kalanchoe pinnatum* leaf extracts. The experimental period lasted 28 days, during which behavioral test like the Morris water maze was performed to assess cognitive function. Biochemical analyses were conducted to evaluate oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), and catalase as well as antioxidant activities like Glutathione (GSH). The weight of the animal was also measured before and after the administration. The results demonstrated that $AlCl_3$ exposure caused significant oxidative stress, and cognitive impairments. Supplementation with *Kalanchoe pinnatum* leaf extracts markedly reduced MDA levels, enhanced SOD, GSH and catalase activities. Behavioral assessments indicated significant improvements in spatial learning and memory retention in extract-treated groups compared to the $AlCl_3$-only group. These findings suggest that *Kalanchoe pinnatum* leaf extracts exhibit neuroprotective effects through their antioxidant and anti-inflammatory properties, highlighting their therapeutic potential in mitigating aluminum chloride-induced hippocampal toxicity and associated cognitive dysfunction.

Keywords: Aluminum chloride, Biochemical analyses, Behavioral assessments, Hippocampal Toxicity, *Kalanchoe pinnatum*, Wistar Rats

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Introduction

Aluminum, a pervasive environmental element, is increasingly recognized for its potential neurotoxic effects, particularly in the context of chronic exposure (Banks & Kastin, 1989). The accumulation of aluminum in the brain has been implicated in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, primarily due to its ability to induce oxidative stress, neuroinflammation, and disruption of neurotransmitter systems (Becaria et al., 2002; Campbell et al., 2004). One of the commonly used compounds to model aluminum-induced neurotoxicity in experimental studies is aluminum chloride ($AlCl_3$), which has been shown to impair cognitive functions and cause significant biochemical alterations, particularly in the hippocampus—a brain region critical for learning and memory(Exley, 2013).

Aluminum chloride ($AlCl_3$) is an inorganic compound widely used in various industrial and chemical processes, including water treatment, as a catalyst in organic synthesis, and in the production of aluminum-based products (Exley, 2014). Despite its usefulness, aluminum chloride has garnered significant attention in the field of neuroscience and toxicology due to its potential neurotoxic effects when introduced into biological systems (Exley, 2017; Muralidharan & Swetha, 2023). Aluminum is a non-essential metal in the human body, meaning it does not play a biological role, yet chronic exposure to aluminum-containing compounds has raised concerns about its accumulation in tissues, particularly the brain (Rondeau et al., 2009).

The central nervous system is particularly vulnerable to aluminum toxicity (Braak & Braak, 1991). Once aluminum chloride enters the body, it can cross the blood-brain barrier and accumulate in brain regions such as the hippocampus, cortex, and cerebellum. Research has linked aluminum chloride exposure to cognitive dysfunction and memory deficits, often associated with hippocampal toxicity (Bremar, 2006).

The hippocampus, a brain region essential for learning and memory, is highly susceptible to oxidative damage due to its high metabolic demand and the presence of polyunsaturated fatty acids (Amaral & Lavenes, 2007). Chronic aluminum chloride exposure has been shown to impair synaptic plasticity and cause neuronal loss, factors that contribute to cognitive decline. Furthermore, aluminum chloride has been associated with neuro-inflammatory responses, where the activation of microglia and the release of pro-inflammatory cytokines exacerbate neuronal damage (Anderson et al., 2007).

The neurotoxic effects of aluminum chloride have made it a common agent in experimental models of neurodegenerative diseases, particularly Alzheimer's disease (Dong et al., 2009). Studies have shown that prolonged exposure to aluminum chloride mimics many of the pathological features of Alzheimer's, including beta-amyloid accumulation, tau hyper-phosphorylation, and neuronal apoptosis (El-Rahman, 2003). This has prompted significant interest in investigating potential therapies and interventions that can mitigate aluminum chloride-induced neurotoxicity.

Given the widespread use of aluminum in daily life—from cookware and packaging to pharmaceuticals and personal care products—the risk of chronic low-level exposure to aluminum chloride and its possible long-term effects on the brain is an important public health issue (Muralidharan & Swetha, 2023). Understanding the mechanisms of aluminum chloride toxicity and identifying effective protective agents is crucial for developing strategies to prevent or alleviate the neurological damage associated with aluminum exposure.

The hippocampus is a critical structure within the brain, playing a central role in memory formation, learning, and spatial navigation (Duvernov, 2005). It is intricately involved in the processing of short-term memories into long-term storage and contributes to cognitive functions essential for everyday life (Eichenbaum & Cohen, 2014). Due to its unique position in the brain and its extensive involvement in neural circuitry, the hippocampus is highly vulnerable to various forms of neurotoxicity, which can significantly impair its function and lead to widespread consequences throughout the nervous system (Fanselow & Dong, 2010).

Hippocampal toxicity can result from a range of insults, including exposure to neurotoxic agents such as aluminum and other heavy metals like lead, and mercury (Heckers & Konradi, 2010). Drugs, environmental toxins, etc are other potential neurotoxic agents. These toxic substances can induce neuronal damage through mechanisms such as oxidative stress, mitochondrial dysfunction, excitotoxicity, and neuroinflammation. One of the most prominent outcomes of hippocampal toxicity is the disruption of synaptic plasticity— particularly long-term potentiation (LTP)—which is crucial for learning and memory. Consequently, hippocampal damage often manifests as cognitive impairments, including memory deficits, difficulties with learning, and emotional dysregulation (Kim & Diamond, 2002). Oxidative stress is a key driver of hippocampal toxicity, as the hippocampus is particularly rich in oxygen-dependent processes and contains high levels of polyunsaturated fatty acids, which are prone to peroxidation (Anderson et al., 2007). Reactive oxygen species (ROS) generated during toxic insults can cause lipid peroxidation, protein oxidation, and DNA damage, leading to neuronal death and the degradation of hippocampal structure and function. This oxidative damage is frequently compounded by the activation of neuro-inflammatory pathways, in which the release of pro-inflammatory cytokines exacerbates neuronal injury and contributes to the progression of neurodegenerative diseases (Duvernov, 2005).

The impact of hippocampal toxicity extends far beyond localized damage, as the hippocampus is a key hub within the larger neural network (Fanselow & Dong, 2010). Disruption of hippocampal function can lead to widespread changes in brain activity, contributing to cognitive decline, mood disorders such as depression and anxiety, and an increased risk of neurodegenerative conditions such as Alzheimer's disease. The interplay between hippocampal damage and other brain regions further underscores the critical role of the hippocampus in maintaining overall nervous system health.

Given the importance of the hippocampus in both cognitive and emotional processing, understanding the mechanisms underlying hippocampal toxicity is essential for developing therapeutic strategies to protect the brain from neurotoxic insults (Kim & Diamond, 2002). Efforts to mitigate hippocampal toxicity often focus on antioxidant therapies, anti-inflammatory treatments, and neuroprotective agents that can shield neurons from the damaging effects of toxic exposures. By preserving hippocampal integrity, these interventions hold promise for reducing the broader impact of neurotoxicity on the nervous system and maintaining cognitive health (Anderson et al., 2007).

Kalanchoe pinnatum, commonly known as the "miracle leaf" or "life plant," is a medicinal plant renowned for its broad spectrum of pharmacological properties, including antioxidant, anti-inflammatory, and neuroprotective effects (Akinmoladun et al., 2007). Traditionally used in various cultures for treating wounds, infections, and inflammatory conditions, recent scientific investigations have begun to explore its potential role in mitigating neurodegenerative processes (Bhandari et al., 2021). The bioactive compounds in *Kalanchoe pinnatum*—such as flavonoids, phenols, and triterpenoids—are believed to contribute to its

antioxidative and anti-inflammatory activities, making it a promising candidate for protecting the brain against toxic insults. This study aimed to investigate the effect of *Kalanchoe pinnatum* on the biochemical parameters in the hippocampus of Wistar rats subjected to aluminum chloride-induced toxicity (De Araujo et al., 2018).

Materials and Method

Plant Collection and Authentication

Freshleaves of *Kalanchloe pinnatum* plant were obtained harvested from Krakrama, a community in Asari Toru local government Area of Rivers State. The plant materials were thoroughly washed with distilled water to remove all dust particles and dirt. The *Kalanchloe pinnatum* was identified and authenticated by Dr. (Mrs.) M. G. Ajuru of the Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University and assigned a herbarium numbers RSUPbHO177.

Extraction of *Kalanchoe pinnatum*

The leaves of the plant was left to dry at room temperature between 32-37°C after collection and cleaning until they attained a constant weight. The extraction method used was adopted from Womeni et al. (2013), which is the cold maceration extraction, with little adjustments. The dry plant material was ground to powder using a milling machine. The powdered material weighing of known weight was macerated in a hydro-ethanolic solution made up of known volume in a glass jar with intermittent agitation for 48 hours at 35°C with fresh replacement of solvent after every 24 hours. The concoction was then filtered using Whatman NO. 1 filter paper. The filtrate was then concentrated using a water bath at 40°C. The resultant extract obtained was kept safe at room temperature until it was needed for use in the study.

Experimental Animal Procurement and Care

Twenty (20) Male albino rats weighing between 150 to 220g was used for the study. The animals were obtained from the Animal house of the College of Basic Medical Science, University of Port Harcourt and were housed in well-ventilated cages containing wood shavings for bedding. The animals were allowed to acclimatize for 7 days and were fed with standard grower's mash (UAC Vital Feed produced by Grand Cereals, Jos, Nigeria) and tap water *ad libitum* prior to experimentation. They were maintained under normal environmental temperature (26±2°C) with a normal 12:12 h dark/light cycle. The room was cleaned and disinfected daily and soiled wood shavings was also replaced daily. The feed and water containers was washed on a daily basis as well. Each rat was marked for identification. The experiments wasconducted in accordance with the internationally accepted principles for Laboratory Animal use and care as found in the US guidelines (NIH publication No. 85-23, revised in 1985).

Experimental Design

Twenty (20)) male albino rats were divided into 4 groups (groups I – IV) of 5 rats per group (n=5). Rats in group 1 (normal control) were given feed and water only for 28 days. Rats in group 2 (Aluminum Chloride only) were given feed, water and Aluminum Chloride at a dose level of 100mg/kg BW daily for 28 days. Group 3 were intermittently administered low Dose of *K. pinnatum* at xxxx and 100mg/kgBW of Aluminum Chloride for 28 days and also fed while Group 4 were given feed, water and intermittently administered high Dose of *K. pinnatum* at xxxx and 100mg/kgBW of Aluminum Chloride for 28 days. All administrations

were done Orally using a Galvage. The body weight of the animals wastaken before and after the administrations and were used to assess the general effect of the treatments on the animal.

Behavioural Studies

Studies on behavior was used to evaluate neurological traits and occurrences like memory loss. The behavioral tests carried out during the study was Morris water maze.

Morris Water Maze

The Morris water maze (MWM) test was used to test for spatial learning and memory. The method of Morris (1984) was adopted with some modifications. The test was comprised of a black circular pool of known height and diameter. The pool was filled with water to a known depth and the temperature was maintained at 26°C. The pool wasdivided into four (4) quadrants and a platform of diameter was submerged 2cm below the surface of one of the quadrants.

Prior to the actual test, the animals were subjected to a total of 20 trials in three (3) consecutive days to enable them locate the hidden platform for easy escape. During the actual test, the water was made opaque by adding Cowbell powdered milk to conceal the platform. Facing the wall of the pool, the animal was gently placed in the water and observed physically from a distance as it swims to locate the hidden platform in 60secs.

The animal was tested to see if they could find the escape platform through spatial memory recognition. Performance was measured by the amount of time it takes to reach the platform (called "latency"), which served as a representation of learning.

Biochemical Analysis

After the experimental period, the rats were sacrificed under anesthesia, and the hippocampus was rapidlydissected out, weighed, and homogenized in ice-cold phosphate-buffered saline (PBS) (pH 7.4) using a Glass-Teflon homogenizer to obtain a 10% (w/v) homogenate. The homogenate was then centrifuged at 10,000 x g for 15 minutes at 4°C, and the supernatant was collected for biochemical analysis of Oxidative Stress Markers like Malondialdehyde (MDA) levels, Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) Activities.

Measurement of Malondialdehyde (MDA) Levels

Principle: Malondialdehyde (MDA) is a byproduct of lipid peroxidation, and its levels are commonly measured as an indicator of oxidative stress. Thiobarbituric acid reactive substances (TBARS) assay was used to quantify MDA levels. In this assay, MDA reacts with thiobarbituric acid (TBA) to form a pink chromogen, which can be measured spectrophotometrically. 2.5 mL of trichloroacetic acid (10%) and 1 mL of TBA (0.67%) was added to 0.5 mL of the hippocampal supernatant and thoroughly mixed. The mixture was incubated in a boiling water bath (90-100°C) for 15 minutes. After cooling the samples on ice, it was centrifuged at 3,000 x g for 10 minutes to remove any precipitates. The absorbance of the supernatant wasthen measured at 532 nm using a spectrophotometer. finally, the MDA concentration was calculated using the molar extinction coefficient of the MDA-TBA complex ($\varepsilon = 1.56 \times 10^5 M^{-1}cm^{-1}$) and the MDA levels were expressed as nano moles of MDA per milligram of protein.

Measurement of Superoxide Dismutase (SOD) Activity

Principle: Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the dismutation of the superoxide anion into hydrogen peroxide and oxygen. SOD activity can be measured based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome. A reaction mixture containing 0.1 mL of hippocampal supernatant, 0.1 mL of ethylenediaminetetraacetic acid (EDTA, 1.0 mM), 0.5 mL of carbonate buffer (50 mM, pH 10.2), and 0.1 mL of epinephrine (10 mM) was prepared. Immediately after adding epinephrine, the increase in absorbance at 480 nm was measured every 30 seconds for 5 minutes using a spectrophotometer. Then the SOD activity was calculated by determining the percentage inhibition of epinephrine auto-oxidation compared to a control (without the enzyme). SOD activity was expressed as units per milligram of protein, where one unit is defined as the amount of enzyme required to inhibit the rate of auto-oxidation of epinephrine by 50%.

Measurement of Catalase Activity

Principle: Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. Catalase activity is measured by monitoring the decrease in absorbance of hydrogen peroxide at 240 nm. A reaction mixture containing 1.95 mL of phosphate buffer (50 mM, pH 7.0), 1.0 mL of hydrogen peroxide (30 mM), and 0.05 mL of hippocampal supernatant will be prepared. Immediately, the decrease in absorbance at 240 nm will be measured for 1 minute using a spectrophotometer. The catalase activity based on the rate of decrease in absorbance, using the molar extinction coefficient for hydrogen peroxide (ε = 43.6 M^−1cm^−1) was be calculated. Catalase activity was expressed as micromoles of hydrogen peroxide decomposed per minute per milligram of protein.

Measurement of Glutathione (GSH) Activity

Principle: Glutathione peroxidase (GPx) is an enzyme that reduces hydrogen peroxide and organic hydroperoxides using reduced glutathione (GSH) as a substrate, forming oxidized glutathione (GSSG). GPx activity is measured by monitoring theoxidation of GSH, coupled to the reduction of GSSG by glutathione reductase in the presence of NADPH.

A reaction mixture containing 1.0 mL of phosphate buffer (50 mM, pH 7.0), 0.1 mL of GSH (10 mM), 0.1 mL of NADPH (1 mM), 0.1 mL of glutathione reductase (1 U/mL), and 0.1 mL of hippocampal supernatant was prepared by adding 0.1 mL of hydrogen peroxide (1 mM). The decrease in absorbance of NADPH was measured at 340 nm for 3 minutes using a spectrophotometer and GPx activity was calculated based on the rate of decrease in absorbance, using the molar extinction coefficient for NADPH (ε = 6.22 x 10^3 M^−1cm^−1) expressed as micromoles of NADPH oxidized per minute per milligram of protein.

Statistical Analysis

Data obtained from the experiments was analyzed using Statistical Package for Social Science (IBM SPSS, Version 26.0). Data will be presented in descriptive statistics (mean and standard error of mean [SEM]) followed by inferential statistics (analysis of variance (ANOVA), followed by A Tukey post hoc test for multiple comparisons). The level of significance was set at $p<0.05$. Graphs and histograms was plotted using Microsoft Excel Version 2019.

Ethical Consideration

This study was carried out in accordance with the ethical guidelines of the National Health Research and Ethics Committee of Nigeria. An ethical clearance was obtained from the

research and ethics department of University of Port Harcourt before the commencement of the research.

Results

Effect of *K. pinatum* on the body weight of Aluminum Chloride induced toxicity.

The mean ± SD of the weight of the experimental animals showed a decrease in the mean weight of the animals fed with $AlCl_3$ with mean ± SD of 94.60 ± 13.48 when compared to their initial body weight (114.20 ± 13.42). At the conclusion of the testing period, the body weight of the control group has increased from a mean value of 105.20 ± 11.26 to a mean value of 123.80 ± 3.19. The weight of animals treated with low and high dose *K. pinnatum* also increased. When compared with the $AlCl_3$ treated group, the differences are quite significant across the different groups.

Table 1. Effect of *K. pinnatum* on the body weight of Aluminum Chloride induced toxicity before and after treatment

Groups	Before	After
Control	105.20 ± 11.26	123.80 ± 3.19#
$AlCl_3$	114.20 ± 13.42	94.60 ± 13.48*
Low Dose *K. Pinnatum* +$AlCl_3$	115.20 ± 9.01	134.60 ± 12.66#
High Dose, *K. Pinatum*+$AlCl_3$	113.20 ± 4.87	131.60 ± 6.58#

Mean ± standard deviation (SD) is used to represent values. At $p<0.05$, the *value differs substantially from the Control; at $p<0.05$, the #value differs significantly from the AlCl3 control group.

Effects of *K. pinnatum* on Oxidative stress makers of Aluminum Chloride induced Wistar Rats

There was a reduction in the level of GSH when $ALCl_3$ was induced with mean ± SD of 0.79 ± 0.72. Low dose showed an increment in the GSH level with mean ± SD of 1.40 ± 1.28 and also high dose with mean ± SD of 2.15 ± 1.34. The same trend is applicable to CAT and SOD which shows a reduction in the $AlCl_3$ (1.15 ± 1.05 and 0.19 ± 0.18, respectively) group when compared to the control group (3.20 ± 0.06 and 0.45±0.40, respectively). Subsequently, there is an increase when treated with Low Dose (0.53 ± 1.40 and 0.20 ± 1.18) and also a higher increase when treated with high dose (2.20 ± 1.21 and 0.30 ± 0.17). MDA showed an increase in the $AlCl_3$ group with mean ± SD of 0.29 ± 0.27 respectively, when compared to the control with mean ± SD of 0.26 ± 0.35. Low dose shows a decrease when compared to the $AlCl_3$ group and High dose shows an increase when compared to the low dose group.

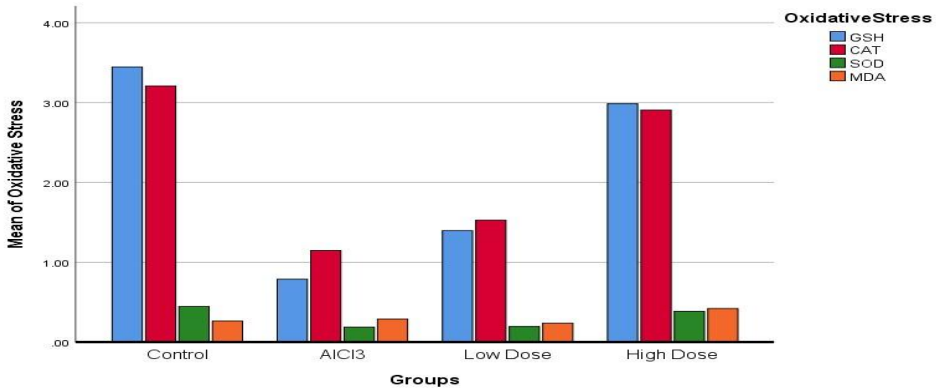


Figure 1. Effects of *K. pinnatum* on Oxidative stress makers of Aluminum Chloride-induced Wistar Rats.

Effect of *Kalanchoe pinnatum* on Spatial Learning and Memory of Aluminum chloride induced toxicity in Wistar rats using Morris Water Maze.

The escape latency period was used to assess learning and memory status in the Morris Water Maze also called forced swim test, as indicated in Table 4.2. The test showed that the group treated with $AlCl_3$ alone had a significantly longer escape latency duration than the control group across the three trials ($P<0.05$). This finding demonstrated that $AlCl_3$ lowers the long-term memory index The also table shows a significant decrease in the Low Dose and High dose of *K.pinnatum* + $AlCl_3$ with mean ± SD of 117.13 ± 16.12 and 117.73 ± 17.75 respectively, when compared to the $AlCl_3$ group as shown in table 3.

Table 2. Effect of *Kalanchoe Pinnatum* on Spatial Learning and Memory of Aluminum chloride induced toxicity in Wistar rats.

Groups	Trial 1 (secs)	Trial 2 (secs)	Trial 3 (secs)
Control	115.20 ± 9.55	115.20 ± 9.55#	101.40 ± 18.86#
AlCl3	122.60 ± 1.82*	164.40 ± 22.33*	162.40 ± 32.11*
Low Dose	123.60 ± 2.30	121.80 ± 13.10#	106.00 ± 22.33#
High Dose	123.40 ± 2.70	127.40 ± 8.99#	102.40 ± 21.778#

Mean ± standard deviation (SD) is used to represent values. At $p<0.05$, the *value differs substantially from the Control; at $p<0.05$, the #value differs significantly from the AlCl3 control group.

Table 3. Effect of *Kalanchoe Pinnatum* on Spatial Learning and Memory ofAluminum chloride induced toxicity in Wistar rats.

Groups	Mean ± SD (Secs)
Control	110.60 ± 14.11#
$AlCl_3$	149.80 ± 28.90*
Low Dose + $AlCl_3$	117.13 ± 16.12#
High Dose + $AlCl_3$	117.73 ± 17.75#

Mean ± standard deviation (SD) is used to represent values. At $p<0.05$, the *value differs substantially from the Control; at $p<0.05$, the #value differs significantly from the AlCl3 control group.

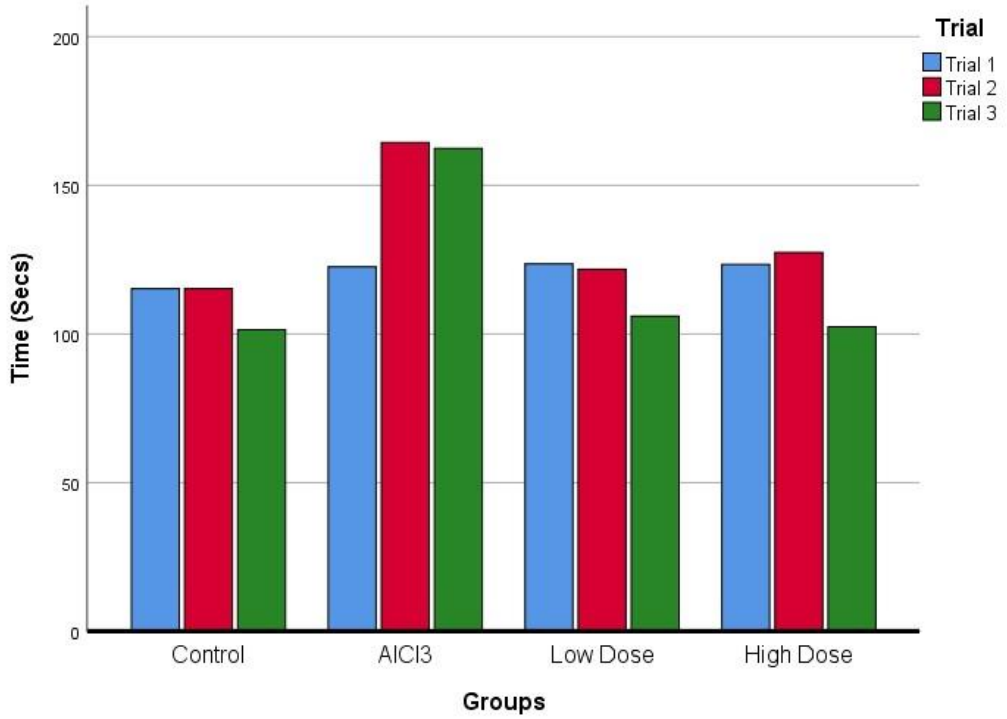


Figure 2. Bar chart showing the effects of *Kalanchoe Pinnatum* on Spatial Learning and Memory of Aluminum chloride induced toxicity in Wistar rats

Discussion

The hippocampus is critical for learning, memory, and other cognitive functions, and its damage can result in severe neurodegenerative outcomes. Aluminum chloride ($AlCl_3$) has been widely used in experimental models to induce neurotoxicity, mimicking Alzheimer's-like conditions through oxidative stress, inflammation, and disruption of biochemical homeostasis (Exley, 2017). In recent years, the potential neuroprotective effects of natural plant extracts, such as *Kalanchoe pinnatum*, have been explored due to their antioxidant, anti-inflammatory, and neurotrophic properties. Various studies have highlighted that plant extracts can improve antioxidant activity, modulate inflammatory responses, and promote tissue preservation, which are crucial for neuroprotection against central nervous system injuries (Moura et al., 2023). Specifically, *Kalanchoe pinnatum* has been noted for its ability to enhance neuronal health through these mechanisms, aligning with broader research on the neuroprotective potential of medicinal plants (Duarte et al., 2023).

Oxidative stress plays a significant role in the hippocampal toxicity induced by aluminum chloride ($AlCl_3$), which is characterized by increased lipid peroxidation and diminished antioxidant defense systems. In this study, it was observed that administering *Kalanchoe pinnatum* leaf extract notably reduced malondialdehyde (MDA) levels, a marker for lipid peroxidation, in comparison to untreated rats exposed to $AlCl_3$. This suggests that *K. pinnatum* may offer protective effects against oxidative damage in the hippocampus caused by aluminum exposure, highlighting its potential as a therapeutic agent in mitigating oxidative stress-related neuronal toxicity. This indicates that the extract mitigates free radical-induced damage, potentially due to its high flavonoid and phenolic content, which scavenges reactive oxygen species (ROS) which is in line with earlier studies. (De Araújo *et al.*, 2018, Ramon *et al*., 2023). Simultaneously, a significant restoration of endogenous antioxidants, such as

superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), was observed in this study, suggesting an enhancement of the brain's intrinsic defense mechanisms which is in line with a study by Bhandari *et al.* (2021) which evaluated the antioxidant capacity of *K. pinnatum* extracts, demonstrating significant free radical scavenging activity. In the study, the methanolic extract showed a notable ability to inhibit DPPH radicals, indicating its potential to neutralize oxidative stress in neuronal tissues (Bhandari *et al.*, 2021).

In another study, the extract was found to increase catalase activity and lower malondialdehyde (MDA) levels in human skeletal muscle myoblasts under oxidative stress, suggesting a protective effect against oxidative damage that could be relevant for hippocampal neurons (Ramon *et al.*, 2023). In experiments involving mice, administration of *K. pinnata* extracts improved memory retention in scopolamine-induced amnesic models. The study reported that higher doses (400 mg/kg) significantly enhanced cognitive performance compared to control groups. This cognitive enhancement is likely linked to the antioxidant properties of the extract, which may reduce neurotoxic effects associated with oxidative stress (Bhandari *et al.*, 2021).

Aluminum chloride ($AlCl_3$) is widely recognized for its neurotoxic effects, leading to various health issues, including weight alterations in animal models. In this study, it was observed that the administration of aluminum chloride actually led to a reduction in body weight of the animals. This is in line with several studies that states that $AlCl_3$ exposure has been associated with oxidative stress, resulting in cellular damage and inflammation. Studies have shown that $AlCl_3$ can lead to significant reductions in body weight and impairments in metabolic functions due to its neurotoxic properties. For instance, $AlCl_3$ administration has been linked to decreased levels of acetylcholinesterase (AChE), which is crucial for maintaining normal neurotransmission and metabolic processes (Usen & Enogieru, 2023). The resulting neuroinflammation and oxidative stress contribute to weight loss and other systemic effects.

The administration of *K. pinnatum extract* actually ameliorated the toxic effect of aluminum chloride by leading to a corresponding increase in body weight of the animals. This is in line with preliminary studies which suggested that *Kalanchoe pinnatum* can enhance antioxidant enzyme activity, potentially mitigating the harmful effects of $AlCl_3$ on body weight and overall health (Muralidharan & Swetha, 2023).

In experimental studies involving Wistar rats, administration of Kalanchoe pinnatum leaf extract alongside $AlCl_3$ has shown promising results. The extract appears to prevent the significant body weight loss typically observed with $AlCl_3$ treatment. For instance, rats treated with Kalanchoe pinnatum exhibited a stabilization or even an increase in body weight compared to those receiving only $AlCl_3$, indicating a protective effect against the toxic agent (Jadhav& Kulkarni, 2023). This suggests that the phytochemicals present in *Kalanchoe pinnatum* may play a crucial role in preserving metabolic functions and preventing the adverse effects of aluminum toxicity.

The study of the effects of *Kalanchoe pinnatum* leaf extract on spatial learning and memory retention, particularly in the context of aluminum chloride-induced toxicity in Wistar rats, offer insights into potential therapeutic interventions for cognitive impairments. In this study, aluminum chloride exposure induced neurotoxicity, leading to deficits in memory and learning capabilities, which was assessed through standardized behavioral tests called Morris Water Maze (MWM).

The neurotoxicity observed is in line with several researches which indicates that exposure to aluminum compounds can lead to oxidative stress, inflammation, and neuronal damage, particularly affecting the hippocampus—a critical region for learning and memory (Narayanan et al., 2009, Safari et al., 2021). Wistar rats exposed to aluminum chloride exhibit significant impairments in spatial learning and memory retention, as evidenced by increased escape latencies and reduced time spent in target zones during MWM tasks (Vorhees& Williams, 2014).

Preliminary studies suggest that *Kalanchoe pinnatum* may enhance cognitive functions by improving synaptic plasticity and reducing neuroinflammation. This is possible because the plant possesses antioxidant activities, anti-inflammatory effects and neurotransmitter modulations. In experiments involving Wistar rats subjected to aluminum chloride toxicity, administration of *Kalanchoe pinnatum* leaf extract has shown promising results. Rats treated with the extract demonstrated improved performance in MWM tasks compared to untreated controls. Specifically, treated rats exhibited shorter escape latencies and increased time spent in the target quadrant during probe trials, indicating enhanced spatial learning capabilities (Tanaka & Taniuchi, 2024). The retention of spatial memory was significantly better in the extract-treated group, suggesting a protective effect against the cognitive decline induced by aluminum chloride(Méndez-López et al., 2009).

Conclusion

The findings suggest that *Kalanchoe pinnatum* leaf extracts possess significant neuroprotective properties, potentially mitigating aluminum chloride-induced hippocampal toxicity in Wistar rats. This effect is mediated through restoration of oxidative balance and improvement of antioxidant defense systems. These results support the therapeutic potential of *K. pinnatum* in managing neurotoxicity and oxidative stress-related disorders.

Additional studies with larger sample sizes and longer treatment durations are recommended to confirm these findings and explore the mechanisms of action. Investigating the efficacy of *K. pinnatum* extracts in human subjects through clinical trials would provide valuable insights into its therapeutic applications. Future research should focus on isolating and characterizing specific phytochemicals responsible for the observed neuroprotective effects.

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