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Original Article

Improvement in Cognition, Memory and Hippocampal CA1 microstructure in Aluminium Chloride-exposed Rats Following Pretreatment with Aqueous *Psidium guajava* Leaf Extract

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ABSTRACT

Reports indicate that humans are commonly exposed to aluminium (Al) to the extent that neuronal death may occur. However, studies show that antioxidant sources can inhibit the toxic effects of Al. P*sidium guajava*, a traditional plant known for its diverse medicinal properties, is reported to be a potential source of natural antioxidants. Hence, this study investigated the activity of aqueous P*sidium Guajava* leaf extract (APGE) against $AlCl_3$-induced neurotoxicity. Thirty Wistar rats were divided into six equal groups as follows: Group A (Control) received 1 ml H_2O/day, Group B received 100 mg/kg body weight (BW)/day of $AlCl_3$ only, Group C received 200 mg/kg BW/day of APGE and 100 mg/kg BW/day of $AlCl_3$, Group D received 400 mg/kg BW/day of APGE and 100 mg/kg BW/day of $AlCl_3$, Group E received 200 mg/kg BW/day of APGE only and Group F received 400 mg/kg BW/day of APGE only, for twenty-eight days. Thereafter, the Novel Object Recognition test was conducted and the rats were sacrificed to harvest the hippocampus for antioxidant enzymes activity, lipid peroxidation, acetylcholinesterase and histological evaluation. Results showed a significant decline in the discrimination index and antioxidant enzymes activity, in addition to elevated lipid peroxidation, increased acetylcholinesterase and severe hippocampal alterations in $AlCl_3$-exposed rats when compared to the control. Conversely, pretreatment of Al-exposed rats with APGE mitigated the effects induced by $AlCl_3$. Altogether, these findings showed that APGE protected against $AlCl_3$-induced hippocampal toxicity, consequently providing novel research evidence on its therapeutic activity against Al.

Keywords: Aluminium, Neurotoxicity, Hippocampus, P*sidium guajava*

INTRODUCTION

Aluminium is a ubiquitous element and the third most prominent in the earth's crust, next to oxygen and silicon (Rahimzadeh *et al.*, 2022). Aluminium is frequently accessible to animal and human populations to the extent that intoxications may occur. Intake of aluminium is by inhalation of aerosols or particles, ingestion of food, water and medicaments, skin contact, vaccination, dialysis and infusions (Igbokwe *et al.*, 2019). Animals and humans living in environments contaminated by industrial wastes may also be exposed to high levels of aluminium (Boran and Iyengar, 2010). Aluminium ion has no physiological role in metabolic states and can be toxic to humans and animals when there is a high accumulation of the metal after natural or unnatural exposure (Igbokwe *et al.*, 2019). Aluminium was considered unsafe for humans after the discovery of higher-than-normal levels in the brain following post-mortem examinations of humans with Alzheimer's disease (Mirza *et al.*, 2017). Toxicosis due to aluminium accumulation in mammalian tissues has been associated with various pathologic effects. Reports indicate that the brain is the most susceptible to the toxic effects of aluminium (Taïr *et al.*, 2016). The mechanisms underlying aluminium-induced neurotoxicity are complex, however, oxidative stress and inflammation are commonly reported (Skalny *et al.*, 2021). Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species (ROS)

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in cells and tissues and the inability of a biological system to detoxify these reactive products (Enogieru and Momodu, 2021a). Antioxidants are a defence mechanism produced by the body to neutralize the effects of ROS and scavenge free radicals, thereby balancing oxidation and antioxidation (Xu *et al.*, 2017). Consequently, it is believed that an increased intake of exogenous antioxidants, even from medicinal plants, would ameliorate the damage caused by oxidative stress through inhibition of the commencement of oxidative chain reaction, thus acting as free radical scavengers (Enogieru and Momodu, 2021a; Fadaka *et al.*, 2019).

Traditional medicine has become more popular due to its richness in natural antioxidants (Enogieru and Momodu, 2021b; Enogieru *et al.*, 2018; Omoruyi *et al.*, 2019). P*sidium guajava* (Guava) belonging to the Myrtaceae family is a unique and traditional plant which is grown due to its diverse medicinal and nutritive properties. It is also revealed to be a potential source of natural antioxidants (Farag *et al.*, 2020). Various parts of this plant have several medicinal properties ranging from antimicrobial, anticancer, antidiarrheal, antiparasitic, antitussive, hepatoprotective, antioxidant, antigenotoxic, antimutagenic, antiallergic, and anti-hyperglycemic effects (Ravi and Divyashree, 2014). P*sidium guajava* leaf extracts and essential oil from the stem and bark can scavenge free radicals and inhibit the formation of hydroxyl radicals (Ravi and Divyashree, 2014). The antioxidant action of P*sidium guajava* has been attributed to quercetin, carotenoids and polyphenols (Naseer *et al.*, 2018; Ravi and Divyashree, 2014). Although its helpfulness encourages further exploration into its effectiveness as a neuroprotective agent, there is a dearth of research evidence on its activity against Aluminium-induced neurotoxicity. Accordingly, this study was designed to investigate such activity; and consequently, results from this study would provide novel research evidence on the protective activity of *Psidium guajava* in the hippocampus of Aluminium-exposed Wistar rats.

MATERIALS AND METHOD

Chemicals and reagents

Normal saline was manufactured by Unique Pharmaceuticals, Sango-Otta, Nigeria, and Aluminium chloride (98 %) by Loba Chemie Pvt. Ltd, Mumbai, India. Other reagents were all of the analytical grades.

Plant extract

Fresh *Psidium guajava* leaves were purchased from the Oba market in the City of Benin, Edo State, and identified at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria with herbarium number UBH-P378. These leaves were air-dried at room temperature and extracted as previously reported by our laboratory (Usen and Enogieru, 2023). Briefly, the leaves were ground through a laboratory mortar and pestle followed by sieving. One kilogram of the fine powder was saturated in 10 litres of distilled water and was extracted for Twenty-four hours. The water extract was collected and filtered using Whatman filter paper No 42 (125 mm) and was thereafter freeze-dried (LGJ-10, SearchEquipment, UK) to obtain a dried powder which was kept at 4 °C until used.

Phytochemical Quantification of extract

Determination of total phenolic content

The total phenolic content in the extract was determined with Folin–Ciocalteu reagent, as previously described (Singleton and Rossi, 1965), with slight modification using tannic acid as a standard. Briefly, 1.0 ml of Folin-Ciocalteu reagent was added to 1.0 ml of extract solution (250 Ug/ml) and mixed thoroughly. After 5 minutes, 15.0 ml Na_2CO_3 (20 %) was added and allowed to stand for 2 hours (Singleton and Rossi, 1965). The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as tannic acid equivalents.

Flavonoid content determination

Total flavonoid content was determined as previously described (Lin and Tang, 2007). Aliquots were prepared by dissolving 0.1 g of extracts in 1 mL deionized water. 0.5 mL of sample solution was taken out and was mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10 % aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$), 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of deionized water. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mgQe/g). Absorbance was determined in triplicate for all extracts, respectively.

Estimation of total Saponin content

The estimation of total saponins content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications, as previously described (Senguttuvan *et al.*, 2014). About 50 µL, from the previously prepared aliquots of sample extract, was added with 250 µL of distilled water. To this, about 250

μL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added (Senguttuvan *et al.*, 2014). Then 2.5 mL of 72 % sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 °C for 10 minutes (Senguttuvan *et al.*, 2014). Diosgenin was used as a standard for calculating the saponin content (mgDe/g). Absorbance was determined in triplicate for all extracts, respectively.

Estimation of alkaloid content

The total alkaloid content was measured as previously described (Anaduaka *et al.*, 2013). Briefly, 5 g of the extract was weighed into a 250 mL beaker and 100 mL of 20 % acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1 % ammonia solution, dried and weighed.

$$\text{Alkaloid (\%)} = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

All samples were analyzed in triplicates.

Estimation of tannin content

Exactly 0.20 mL, from the previously prepared aliquots of sample extract, was added to 20 mL of 50 % methanol and placed in a water bath at 77 ˚C – 80 ˚C for 1 hour and shaken (Senguttuvan *et al.*, 2014). The extract was quantitatively filtered using a double-layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17 % Na_2CO_3 were added and mixed (Senguttuvan *et al.*, 2014). The mixture was allowed to stand for 20 minutes. A series of standard tannic acid solutions were prepared in methanol and their absorbance as well as samples were read after colour development on a spectrophotometer at a wavelength of 760 nm (Senguttuvan *et al.*, 2014). Total tannin content was calculated from a calibration curve as previously reported (Senguttuvan *et al.*, 2014).

Animals

Wistar rats for the study were bred at the Animal House, Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria. They were kept in polypropylene cages at room temperature. The rats were fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and had free access to water throughout the entire study period of twenty-eight days. Protocols for this experiment were per the guide for the care and use of laboratory animals approved by the Research Ethical Committee of the College of Medical Sciences, University of Benin with approval number CMS/REC/2021/269.

Research design

A total of thirty adult Wistar rats weighing between 150g and 180g were used for this study. They were randomly assigned into six groups (A-F) of five rats each, after acclimatization to animal house conditions for two weeks, with free access to feed and water. All administrations were consumed orally with the help of an oral gavage throughout the entire study period of twenty-eight days. Group A (Control) received 1 ml H_2O/day, Group B (Al) received 100 mg/kg body weight (BW)/day of $AlCl_3$ only, Group C (APGE1 + Al) received 200mg/kg BW/day of aqueous *Psidium guajava* Leaf extract (APGE) and 100 mg/kg BW/day of $AlCl_3$, Group D (APGE2 + Al) received 400 mg/kg BW/day of APGE and 100 mg/kg BW/day of $AlCl_3$, Group E (APGE1) received 200 mg/kg BW/day of APGE only and Group F (APGE1) received 400 mg/kg BW/day of APGE only. All rats were pretreated with APGE one hour before the administration of Aluminium chloride. After 28 days, the Novel Object Recognition (NOR) neurobehavioural test was carried out.

Evaluation of Neurobehavioral Activity

The Novel Object Recognition (NOR) test, commonly used to assess short-term and long-term memory in rodents, was done as previously described (Enogieru and Omoruyi, 2022; Enogieru and Inegbedion, 2022). Briefly, on the 28th day of the experiment, each rat explored the apparatus for 2 minutes, while on day 29 (test day), two sessions (T1 and T2) of 3 minutes each was allowed (Enogieru and Inegbedion, 2022). In T1 (trial), two similar objects (FO1 and FO2) were placed at opposite corners of the apparatus. Thereafter, rats were left to individually explore both identical objects (Enogieru and Inegbedion, 2022). At the end of T1, rats were returned to their cages and a 1-hour interval was given before T2. In T2 (real test), a new object (NO) was used to replace FO2, and each rat was left to explore FO1 and NO (Enogieru and Omoruyi, 2022). Thereafter, the total time spent in exploring FO1 and FO2 (in T1), and that spent in exploring FO1 and NO (in T2) was recorded (Enogieru and Omoruyi, 2022). The discrimination index (DI) was calculated as NO−FO1/NO + FO1. The

apparatus was cleaned with alcohol and the position of the two objects during T2 was changed randomly to avoid place preference and the influence of olfactory stimuli.

Antioxidant Enzymes and Lipid Peroxidation Assessment
Following the sacrifice of rats under low-level chloroform anaesthesia before cervical dislocation, the brains of experimental rats were removed and the hippocampus was dissected for biochemical and histological investigations. The harvested hippocampus was washed twice in cold phosphate-buffered saline (PBS), homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and centrifuged at 10,000 g for 10 minutes at 4 °C (Enogieru and Momodu, 2022; Enogieru and Iyoha, 2023). The supernatant was collected and assessed for Malondialdehyde (MDA) (Guttridge and Wilkins, 1982), Catalase (CAT) (Cohen *et al.*, 1970), Superoxide Dismutase (SOD) (Misra and Fridovich, 1972) and Glutathione (GSH) (Ellman, 1959).

Estimation of Acetylcholinesterase (AChE) Activity

AChE activity was estimated as previously described (Ellman *et al.*, 1961). Briefly, a reaction mixture comprised of 2.35 ml sodium phosphate buffer (0.1 M, pH 7.4), 0.2 ml 5,5-dithiobis-2-nitrobenzoic acid (10 mM), 0.2 ml triton X100 (0.013%), 0.05 ml tissue homogenate and 0.2 ml of 10 mM acetylthiocholine (Ellman *et al.*, 1961). AChE activity was measured spectrometrically at 412 nm and results were expressed as nmol/ACh hydrolyzed/min/mg protein.

Histological Assessment
The hippocampi were fixed in 10 % buffered formal saline for 72 hours, and processed through the Hematoxylin and Eosin staining methods as previously described (Drury and Wallington, 1980).

Statistical analysis
One-way analysis of Variance (ANOVA) and Tukey's post hoc test were utilized to ascertain statistical differences with Graph Pad Prism Software, Version 7. Results are expressed as mean ± Standard Error of Mean (S.E.M.) and P value <0.05 was taken as statistically significant.

RESULTS

Quantitative Phytochemical Screening of APGE
The quantity of phytochemicals present in APGE is shown in Table 1. It was observed that saponins were the most abundant.

Table 1: Quantitative estimation of phytochemicals in APGE

Phytochemicals	Amount (mg/g)
Total Phenols	12.68 ± 0.25
Total Flavonoids	24.19 ± 1.28
Total Tannins	16.94 ± 0.10
Total Saponins	104.8 ± 2.25
Total Alkaloids	3.38 ± 0.08

Effect of APGE on Cognition and Memory
Table 2 shows the findings on cognition and memory from the Novel Object Recognition Test. In Al-exposed rats (group B), a significant decrease ($p < 0.05$) was observed in the mean exploration times for FO1, FO2, NO and total exploration times, T1 and T2, when compared to control. However, in groups C (Al + APGE1) and D (Al + APGE2), a significant increase ($p < 0.05$) was observed in the mean exploration time for NO when compared to Al-exposed rats (group B). Similarly, whereas a significant decrease ($p < 0.05$) was observed in the discrimination index in Al-exposed rats (group B) when compared to control, a significant increase ($p < 0.05$) was observed in groups C (Al + APGE1) and D (Al + APGE2) when compared to Al-exposed rats (group B). No significant differences ($p > 0.05$) were observed between groups E (APGE1) and F (APGE2) when compared to control.

Table 2: Novel object recognition parameters across experimental groups

Parameters	Control	Al	APGE1 + Al	APGE2 + Al	APGE1	APGE2
FO1 (Trial)	33.25 ± 4.54	14.75 ± 4.03#	25.0 ± 1.63	24.0 ± 0.71	26.5 ± 1.71	32.0 ± 1.87
FO2 (Trial)	31.75 ± 1.75	10.40 ± 3.50#	22.75 ± 2.63	21.75 ± 2.02	33.50 ± 0.96	25.00 ± 4.51
FO1	12.80 ± 2.01	9.40 ± 1.63	11.80 ± 2.40	12.40 ± 3.11	11.00 ± 1.70	12.00 ± 2.37
NO	51.80 ± 3.48	13.00 ± 2.24#	35.60 ± 3.75*	33.00 ± 5.43*	36.20 ± 4.35	45.40 ± 3.23
T1	65.00 ± 6.10	22.20 ± 7.68#	47.75 ± 3.59	45.75 ± 1.97	60.00 ± 2.08	57.00 ± 6.12
T2	64.60 ± 4.99	22.40 ± 3.83#	47.40 ± 4.89	45.40 ± 8.25	47.20 ± 5.45	57.20 ± 4.06
DI	0.612 ± 0.05	0.169 ± 0.03#	0.511 ± 0.08*	0.486 ± 0.07*	0.538 ± 0.05	0.588 ± 0.07

and * represent *p*< 0.05 following comparison to the control and Al respectively. (FO1 – Familiar object 1; FO2 – Familiar object 2; NO – Novel object; T1 – Total exploration time 1; T1 – Total exploration time 2; DI - Discrimination index)

Activity of APGE on Hippocampal Antioxidant enzymes, Lipid Peroxidation and AChE

A significant decrease ($p < 0.05$) in SOD and GSH was observed in Al-exposed rats (group B) when compared to control (Table 3). However, a significant increase ($p < 0.05$) in SOD, CAT and GSH was observed in group C (Al + APGE1) and SOD in group D (Al + APGE2) when compared to group B. Also, a significant increase ($p < 0.05$) in MDA and AChE was observed in group B when compared to control. Conversely, a significant decrease ($p < 0.05$) in MDA and AChE was observed in groups C (Al + APGE1) and D (Al + APGE2) when compared to group B. No significant differences ($p > 0.05$) were observed between groups E (APGE1) and F (APGE2) when compared to control.

Activity of APGE on histology of the hippocampus

Plate 1A shows normal hippocampus histology. Plate 1B shows atrophy and vacuolation of astrocytes and pyramidal cells due to the administration of aluminium. Plates 1C and 1D show relatively normal structure of pyramidal cells and very mild astrocyte vacuolation following comparison to Al-exposed rats in group B. Plates 1E and 1F revealed normal structure of pyramidal cells and astrocytes comparable to that of the rats in control group A.

Table 3: Antioxidant Enzymes, Lipid Peroxidation and AChE activity in the hippocampus across experimental groups.

Parameters	Control	Al	APGE1 + Al	APGE2 + Al	APGE1	APGE2
SOD (U/mg)	66.8 ± 4.49	18.1 ± 1.36#	41.1 ± 2.34 *	35.2 ± 2.84*	61.0 ± 3.49	54.1 ± 2.55
CAT (U/mg)	29.4 ± 1.93	14.2 ± 1.07	23.7 ± 1.65 *	18.9 ± 1.52	28.9 ± 2.38	28.5 ± 1.37
GSH (µM)	46.1 ± 2.26	26.1 ± 1.82#	38.8 ± 1.67 *	32.9 ± 1.69	44.5 ± 2.23	42.6 ± 2.19
MDA (mmol/mg)	40.4 ± 3.93	148.1 ± 12.34#	60.7 ± 7.79 *	66.4 ± 4.07*	41.4 ± 5.26	43.2 ± 4.09
AChE (ìmol/mg)	28.9 ± 4.29	166.8 ± 18.26#	87.3 ± 7.82 *	68.2 ± 6.16*	33.8 ± 1.97	32.3 ± 4.71

and * represent p< 0.05 following comparison to the control and Al respectively.

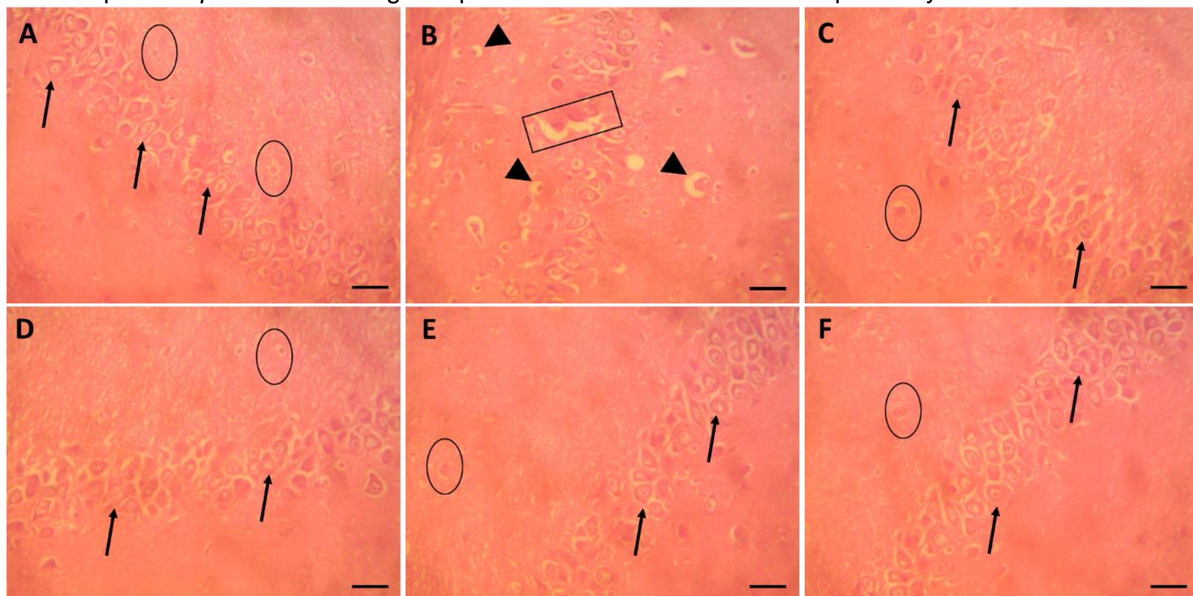

Plate 1: Representative histology of the hippocampus CA1 in experimental rats (A) Normal structure of pyramidal cells (arrows) and astrocytes (circles). (B) Atrophied and vacuolated astrocytes (arrowheads) and degenerated pyramidal cells (box). (C-F) Relatively normal structure of pyramidal cells (arrows) and astrocytes (circles) (H&E x400; Scale bar: 25µm).

DISCUSSION

This study investigated the protective activity of aqueous *Psidium guajava* leaf extract on aluminium-induced hippocampal toxicity in Wistar rats. Reports indicate that behavioural tests that assess the ability to identify a previously presented stimulus form the core of animal models of cognition and memory deficits (Baxter, 2010). One of the most important of these tests includes the novel object recognition (NOR) test which evaluates the animal's behaviour when it is exposed to a novel and a familiar object. (Ennaceur, 2010). When animals are exposed to a familiar and a novel object, they explore the novel more frequently than the familiar one (Ennaceur, 2010). Although the NOR test has become a

commonly utilized model for the investigation into memory deficits, it can be used to assess working memory, attention and inclination for novelty in rodents (Antunes and Biala, 2012). Animal models like the NOR that assess recognition memory have become key useful tools for basic and preclinical research as they allow studying the neural basis of memory (Antunes and Biala, 2012). Moreover, reports indicate that findings from the NOR test are influenced by both hippocampal and cortical lesions (Antunes and Biala, 2012; Clark *et al*., 2000). One of the key indices of recognition in the NOR test is the discrimination index which is defined as the time spent exploring the novel object relative to the total time spent exploring both objects (Antunes and Biala, 2012). Studies indicate that rodents with potent cognitive functions have a higher discrimination index in the NOR tests (Enogieru and Egbon, 2022; Enogieru and Inegbedion, 2022). In this study, aluminium-exposed rats were unable to discriminate between the familiar object and novel object when compared to control; this is in line with previous studies (Chiroma *et al*., 2019; Singh *et al*., 2018). However, pretreatment with APGE attenuated aluminium-induced loss of discrimination in the rats, thus demonstrating the cognition and memory-enhancing effects of *Psidium guavaja*.

Numerous reports demonstrate that antioxidants slow down the rate of oxidation process through their ability to accept or donate electrons and via their direct interaction with free radicals to form less reactive radicals (Garg *et al*., 2022). The body encloses a complex antioxidant defence grid that collectively acts against free radicals including Superoxide dismutase (SOD) and Catalase (CAT) (Ighodaro and Akinloye, 2018). They are regarded as the first line of defence antioxidants due to their indispensable role in the entire antioxidant defence strategy (Ighodaro and Akinloye, 2018). On the other hand, GSH is an essential non-enzymatic antioxidant that acts directly as an antioxidant to protect cells against free radicals and pro-oxidants, and as a cofactor for antioxidant and detoxification enzymes (Averill-Bates, 2023). Findings from this study show that there was a significant reduction in SOD, CAT and GSH in the hippocampus of aluminium-exposed rats when compared to control, thus indicating that aluminium alters antioxidant activities by inhibiting antioxidant enzymes and promoting the generation of reactive oxygen species. Impaired antioxidant defences can be a result of the inhibitory effects of aluminium on various enzymes, which in turn causes the cells to be more susceptible to oxidative insult. This is in line with previous studies (Fernandes *et al.*, 2020; Suryavanshi *et al.*, 2022). Following pretreatment with *Psidium guavaja*, a significant increase was observed in SOD, CAT and GSH in the hippocampus of rats when compared to aluminium-exposed rats, thus indicating that *Psidium guavaja* is able to protect against aluminium-induced oxidative stress. This agrees with previous studies reporting that *Psidium guavaja* is a potent antioxidant (Naseer *et al*., 2018; Usen and Enogieru, 2023). Free radicals are known to induce the lipid peroxidation process in organisms and MDA is one of the final products of polyunsaturated fatty acids peroxidation in cells (Gaweł *et al*., 2004). An increase in free radicals causes overproduction of MDA, and consequently, it is often used as a marker of oxidative stress (Gaweł *et al*., 2004). Results from this study showed a significant increase in hippocampal MDA when compared to the control, thus indicating an induction of oxidative stress in line with previous studies (Fernandes *et al*., 2020; Suryavanshi *et al*., 2022). The significant reduction in hippocampal MDA in the *Psidium guajava* pretreated rats when compared to aluminium-exposed rats signifies the oxidative stress-inhibiting potential of *Psidium guajava*.

Acetylcholine is a neurotransmitter involved in nervous systems, supporting cognitive and physiological functions within the body (Sam and Bordoni, 2022). AChE, a serine hydrolase, primarily functions to degrade acetylcholine and terminate neurotransmission (Richbart *et al*., 2021). Aside from its role in synaptic transmission, AChE is involved in apoptosis, response to stress signals and inflammation (Richbart *et al*., 2021). Whereas the level of AChE is significantly increased in neurodegenerative disorders like Alzheimer's, the level of acetylcholine is reduced due to excessive hydrolysis by AChE (Ojha *et al*., 2022), often leading to learning, memory and cognitive disabilities. The present study demonstrates that pretreatment with *Psidium guajava* significantly reduced AChE activity in the hippocampus when compared to the aluminium-exposed rats, thus further highlighting its cognition and memory-enhancing effects. This agrees with a previous study reporting the potent AChE inhibitory activity of *Psidium guajava* (Bouchoukh *et al*., 2019).

For the histological findings, rats in the control group displayed normal structure of the hippocampus, however, atrophy and vacuolations of astrocytes and pyramidal cells

were observed in the hippocampal CA1 region of rats exposed to aluminium. In previous studies, atrophy of the hippocampus is reported to be an early characteristic of Alzheimer's disease and other dementias, often linked to cognitive decline with the inability to access old memories or make new ones (Van De Pol *et al.*, 2005; Yao *et al.*, 2020). Several previous studies have reported histopathological alterations in the hippocampus due to aluminium exposure and this is further demonstrated in this study (Jadhav and Kulkarni, 2023; Thenmozhi *et al.*, 2015). Pretreatment of rats with *Psidium guajava* mitigated the alterations induced by aluminium, thus highlighting its potent protective effects. Also, administration of *Psidium* guajava-alone to the rats did not alter the microarchitecture of the hippocampus. The protective activities demonstrated in this study against aluminium may be due to the presence of various phytochemicals in the plant such as phenols, flavonoids, tannins, saponins and alkaloids. These phytochemicals have been reported to enhance cognition and memory in experimental models (Bakoyiannis *et al.*, 2019; Libro *et al.*, 2016).

CONCLUSION

Taken together, findings from this study demonstrate that aqueous *Psidium guavaja* leaf extract protects the hippocampus in experimental rats against aluminium-induced, possibly via its potent anticholinesterase and antioxidant effects. Consequently, this study provides novel preliminary research evidence on the possible therapeutic role of aqueous *Psidium guavaja* leaf extract against neurodegenerative disorders linked to cognition and memory.

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