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Original Article
Anticholinesterase and Antioxidant Potential of Aqueous *Phyllanthus amarus* Leaf Extract in Aluminium Chloride-Exposed *Drosophila melanogaster*

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ABSTRACT

Aluminium chloride ($AlCl_3$) toxicity is associated with several neurodegenerative disorders, including Alzheimer's disease, via mechanisms of oxidative stress, impairment of cholinergic neurotransmission, and enhancement of acetylcholinesterase activity. *Phyllanthus amarus* (*P. amarus*) is a widely used medicinal plant with reported antioxidant properties, and accordingly, this study investigated the anticholinesterase and antioxidant potential of *P. amarus* against $AlCl_3$-induced toxicity in *Drosophila melanogaster*. 200 drosophilas were divided into four groups, with 50 per group. The control group was reared on a cornmeal diet, while the $AlCl_3$ group was treated with 40 mM of $AlCl_3$ via their diet. The *P. amarus* group was treated with 2.5 mg of *P. amarus*, while the co-treatment group was co-treated with 40 mM $AlCl_3$ and 2.5 mg of *P. amarus* via their diet for seven days. At the end of the experimental period, negative geotaxis was carried out to evaluate locomotor performance. The drosophilas were thereafter homogenised, and the supernatants were used to assay for acetylcholinesterase (AChE), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) activities. Results showed a significant decrease in the survival rate, climbing activity, SOD, and CAT, as well as a significant increase in MDA and AChE activity in $AlCl_3$-exposed drosophilas. However, co-treatment with *P. amarus* was able to significantly attenuate the toxicity of $AlCl_3$. Taken together, the protective effect of *P. amarus* against $AlCl_3$ is mediated possibly through its anti-cholinesterase and antioxidant properties and could therefore be relevant in the development of novel therapeutic agents useful for the treatment of $AlCl_3$ toxicity and its related disorders.

Keywords
Neurodegeneration, $AlCl_3$ toxicity, *Phyllanthus amarus*, Acetylcholinesterase, *Drosophila melanogaster*

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INTRODUCTION

Aluminium (Al) is a metal with widespread industrial applications. It is used in the manufacture of cooking utensils, roofing sheets, and pharmacological agents, including antacids and antiperspirants (Maya et al. 2016). Al salts, such as aluminium chloride ($AlCl_3$), have been utilised in the investigation of biologically occurring Al compounds and are reportedly linked with several neurodegenerative diseases, including Alzheimer's disease (AD) (Shunan et al. 2021). $AlCl_3$ toxicity has been reported to be associated with impaired locomotor performance, learning, memory, and cholinergic impairment, which are features seen in AD patients (Inneh and Eiya 2023), and an increase in aluminium exposure has also been reported to increase the risk of developing AD by 71% (Wang et al. 2016). $AlCl_3$ induces neurotoxicity and neurodegeneration primarily by triggering oxidative stress, thus affecting a large number of signalling cascades and ultimately causing death. As a cholinotoxic substance, $AlCl_3$ alters acetylcholinesterase (AChE), an enzyme that catalyses the hydrolysis of acetylcholine into choline and acetic acid (Maya et al. 2016).

Acetylcholine is an important neurotransmitter that plays an important role in learning, memory, locomotion, and cholinergic neurotransmission (Haam and Yakel 2017). Studies have shown that the depletion of acetylcholine is responsible for cognitive decline and memory loss (Maurer

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and Williams 2017). One of the most promising approaches for the treatment of AD has been to augment acetylcholine and cholinergic neurotransmission in the brain, and as such, AChE is currently a therapeutic target in the management of AD (Santos et al. 2018). Inhibitors of AChE (donepezil, tacrine, and rivastigmine) increase the endogenous level of acetylcholine and enhance cholinergic transmission in the brain, leading to improvements in memory and other cognitive functions (Moss 2020). However, these drugs have severe side effects like nausea, diarrhoea, vomiting, decreased appetite, dyspepsia, anorexia, muscle cramps, fatigue, insomnia, dizziness, headache, and asthenia (Ohbe et al. 2018), thus leading to the search for natural compounds with antioxidant and AChE inhibitory activities.

Phyllanthus amarus (*P. amarus*) is a small herb belonging to the family Euphorbiaceae (Adedapo et al. 2014). It is a plant well known for its medicinal properties and has been used traditionally to treat hepatitis, tuberculosis, malaria, diabetes, and hypertension (Egbon et al. 2017). The leaves of this plant are reported to contain alkaloids, glycosides, tannins, phenols, saponins, and flavonoids (Enogieru and Omoruyi 2022). *P. amarus* has been reported to possess anti-oxidative, anti-inflammatory, and hepatoprotective properties (Aparupa et al. 2022). Owing to the implication of oxidative stress and impaired cholinergic transmission in several reports and the continuous search for therapeutic agents capable of attenuating $AlCl_3$ toxicity, this study was designed to investigate the anticholinesterase and antioxidant potential of *P. amarus* in $AlCl_3$-exposed *Drosophila melanogaster* (*D. melanogaster*).

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade. $AlCl_3$ (CAS number 7784-13-6) manufactured by GHTECH (China) was purchased from Pyrex Chemicals in Benin City, Edo State. Additional chemicals utilised, such as acetylthiocholine iodide (CAS number 1866-15-5) and 1-chloro-2,4-dinitrobenzene (CDNB) (CAS number 97-00-7), were purchased from Sigma Aldrich (St. Louis, USA).

D. melanogaster Stock and Culture

Two hundred *D. melanogaster* (Harwich strain) 3-5 days old were obtained from the Drosophila Laboratory of the University of Ibadan, Oyo State, Nigeria. The drosophilas were reared on a cornmeal medium that contains 1% w/v agar, 1% w/v brewer's yeast, 1% w/v powdered milk, 2% w/v sucrose, and 0.08% v/w nipagin at room temperature (24 °C) under a 12 h dark/light cycle condition at the Drosophila Laboratory of the Central Research Laboratory, University of Benin, Benin City. The same strain of *D. melanogaster* was utilised during the experiment.

Collection of Plant Material

Phyllanthus amarus (*P. amarus*) leaves were collected from the agricultural farm of the University of Benin and identified at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, with herbarium number UBH-P347.

Plant Extraction

Fresh leaves of *P. amarus* were air-dried, pulverised, weighed, and stored in an airtight container. After this, 1kg of the powdered leaves was extracted with 1.2 L of distilled water for 24 h. 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 and transferred to a refrigerator for storage (at 4 ℃) before use.

Survival Study

The study was approved by the Research Ethics Committee, College of Medical Sciences, University of Benin, with approval number CMS/REC/2023/356. To determine an appropriate dose of *P. amarus* for this study and the effect of $AlCl_3$ toxicity on the survival rate of drosophilas, a 21-day survival study was carried out. Drosophilas (3–5 days old) were shared into three groups (containing 50 drosophilas each), and each group was treated with 40 mM $AlCl_3$, 2.5 mg, and 5 mg of *P. amarus*, respectively, via their diet for 21 days. The drosophilas were observed daily for mortality, and the survival rate was determined by counting the number of dead drosophilas during the 21-day period (Abolaji et al. 2014). The data were subsequently analysed and plotted as a percentage of survival after the treatment period. The data obtained from the survival studies primed the choice of the 2.5 mg diet concentration of *P. amarus* used for the study.

Experimental Layout

D. melanogaster (both genders) were shared into 4 groups, with 50 drosophilas per group. The control group was reared on a cornmeal diet, while the $AlCl_3$ group was treated with 40 mM of $AlCl_3$ via their diet. The *P. amarus* group was treated with 2.5 mg of *P. amarus*, while the co-treatment group was co-treated with 40 mM $AlCl_3$ and 2.5 mg of *P. amarus* via their diet. The concentration of $AlCl_3$ utilised was based on previous studies on aluminium toxicity in *D. melanogaster* (Wu et al. 2012; Adedayo et al. 2020). These drosophilas were treated at room temperature (24 ℃) for seven days. Each experiment was carried out in five replicates (n = 5).

Negative Geotaxis Assay

This assay was used to determine the locomotor activity of drosophila and was carried out as previously reported (Abolaji et al. 2018). Briefly, ten drosophilas from each group were immobilised under ice anaesthesia. They were subsequently placed separately in labelled vertical glass columns (length 15 cm; diameter 1.5 cm). After the recovery from the ice exposure, the bottom of the column was gently tapped, and the drosophilas were allowed to climb. The number of drosophilas that climbed up to and above

the 6 cm mark of the column in 6 sec, as well as those that remained below this mark after this time, was recorded. The scores represent the mean of the number of drosophilas at the top expressed as a percentage of the total number of drosophilas. This procedure was repeated three times at 1-min intervals.

Preparation of Samples for Biochemical Assays
At the end of the 7-day experimental period, the drosophilas (intact whole body) were homogenised, centrifuged, and the supernatants were used for biochemical analysis. The drosophilas were first anaesthetized in ice, thereafter weighed, and then homogenised in 0.1 M potassium phosphate buffer of pH 7.4 (1:10) (Oyetayo et al. 2020). They were later centrifuged at 4,000 g for 10 min at 4 °C, and the supernatants obtained were used for the following biochemical assays: malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and AChE activities.

Determination of Biochemical Indices
SOD Activity
SOD activity was determined by monitoring inhibition of the autoxidation of quercetin as described by Kostyuk and Potapovich (1989). The reaction mixture contained 10 µL of the sample, 15% quercetin, 20 mM phosphate buffer (pH 7.8), 0.08 mM ethylenediamine-tetraacetic acid (EDTA), and 8 mM tetramethylethylenediamine (TEMED). The reaction was monitored for 3 min at 406 nm. The results were expressed as the amount of protein required to inhibit quercetin auto-oxidation (µmol/min/mg protein).

Determination of CAT Activity
CAT activity was determined following the method of Aebi (1984) by mixing 10 µL of sample (1:50 dilution) with 50 mM potassium phosphate buffer (pH 7.0), followed by 300 mM hydrogen peroxide (H2O2). The loss in absorbance of H_2O_2 was monitored for 2 min at 240 nm and was subsequently used to calculate CAT activity, which was expressed as µmol of H_2O_2 consumed per minute per milligram of protein.

Determination of Lipid Peroxidation
Lipid peroxidation was determined according to the method of Ohkawa et al. (1979). The mixture contained 40 mL of the supernatant, 100 mL of 0.67% thiobarbituric acid, 5 mL of 10 mM butyl-hydroxytoluene (BHT), 300 mL of 1% O-phosphoric acid, and 55 mL of distilled water. This was followed by a 45 min incubation time at 90 °C, and the absorbance was measured at 535 nm. The results were expressed as µmol of MDA formed per milligram protein.

Determination of AChE Activity
AChE activity was evaluated following the method described by Ellman et al. (1961). The reaction mixture contained 30 mL of the sample, 1 mM DTNB, 0.1 M of potassium phosphate buffer (pH 7.4), and 0.8 mM acetylthiocholine. This mixture was monitored for 2 min (at 30-s intervals) at 412 nm. The enzyme activity was then evaluated as µmol of acetylthiocholine hydrolysed per min per milligram protein.

Statistical analysis
Statistical analysis was done using the GraphPad Prism 7.0 software. The data was presented as the mean ± SEM. A one-way analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Turkey's multiple comparison post hoc test. $P<0.05$ was taken as statistically significant.

RESULTS

Survival Rate of *D. melanogaster* Exposed to $AlCl_3$ and *P. amarus*
There was a significant decrease in the survival rate of drosophilas exposed to 40 mM of $AlCl_3$ when compared with those of the control ($p< 0.05$). In drosophilas co-treated with 40 mM of $AlCl_3$ and 2.5mg of *P. amarus*, there was a significant improvement in the survival rate of drosophilas ($p< 0.05$) when compared with those treated with $AlCl_3$ only (Fig. 1).

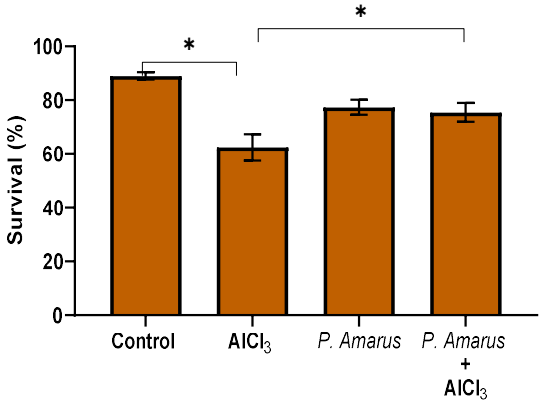

Fig. 1: Effect of aluminium chloride and *P. amarus* on survival rate of drosophilas. Data is presented as mean ±SEM (n = 5, 50 drosophilas/vial). * $p < 0.05$

Effect of $AlCl_3$ and *P. amarus* on Climbing Activity of *D. melanogaster* (Negative Geotaxis)
There was a significant reduction ($p < 0.05$) in climbing/ locomotor activity of drosophilas treated with $AlCl_3$ when compared with control. However, in drosophilas that were concurrently treated with 40 mM of $AlCl_3$ and 2.5 mg of *P. amarus*, there was a significant improvement ($p< 0.05$) in the climbing or locomotor activity when compared with drosophilas treated with $AlCl_3$ only (Fig. 2).

Malonaldehyde (MDA) Concentration of *D. melanogaster* Treated with $AlCl_3$ and *P. amarus*
There was a significant increase ($p < 0.05$) in MDA concentration in drosophilas treated with $AlCl_3$ only. In drosophilas co-treated with $AlCl_3$ and *P. amarus,* a significant reduction ($p<0.05$) was observed in MDA concentration

when compared to drosophilas that received $AlCl_3$ only (Fig. 3).

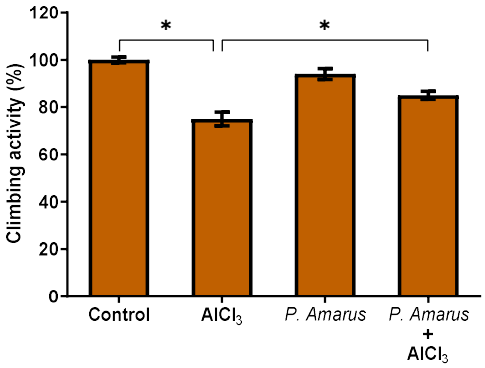

Fig. 2: Effect of $AlCl_3$ and *P. amarus* on climbing activity (negative geotaxis). Data is presented as mean ± SEM (n = 5, 50 drosophilas/vial). * p < 0.05

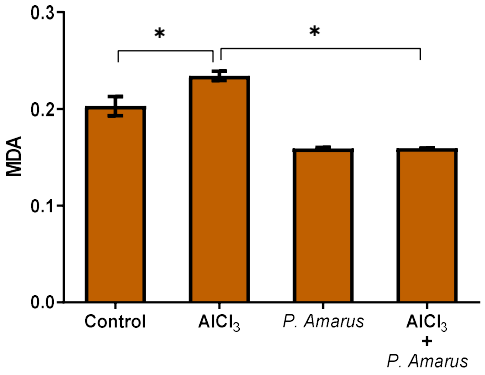

Fig. 3: Effect of $AlCl_3$ and *P. amarus* on MDA concentration. Data is presented as mean ± SEM (n = 5, 50 drosophilas/vial). * P< 0.05

SOD Activity of *D. melanogaster* Treated with $AlCl_3$ and *P. amarus*

There was a significant decrease (p < 0.05) in SOD activity in drosophilas treated with $AlCl_3$ only when compared with those of the control. A significant increase (p < 0.05) in SOD activity was observed in drosophilas that were co-treated with $AlCl_3$ and *P. amarus* when compared with $AlCl_3$-treated drosophilas only (Fig. 4).

CAT Activity of *D. melanogaster* Treated with $AlCl_3$ and *P. amarus*.

$AlCl_3$ significantly decreased (p < 0.05) the CAT activity of drosophilas when compared with that of the control. In drosophilas treated with $AlCl_3$ and *P. amarus*, a significant improvement (p< 0.05) was observed in CAT activity when compared with those treated with $AlCl_3$ only (Fig. 5).

AChE Activity of *D. melanogaster* Treated with $AlCl_3$ and *P. amarus*

There was a significant increase (p < 0.05) in AChE activity in drosophilas treated with 40 mMol of $AlCl_3$ only when compared with those of the control. However, in drosophilas co-treated with $AlCl_3$ and *P. amarus*, there was a significant reduction (p<0.05) in AChE activity when compared to drosophilas that were treated with $AlCl_3$ only (Fig. 6).

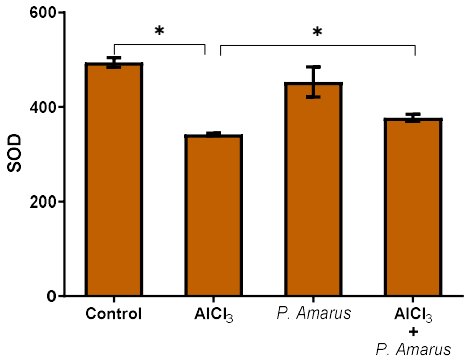

Fig. 4: Effect of $AlCl_3$ and *P. amarus* on SOD activity. Data is presented as mean ± SEM (n = 5, 50 drosophilas/vial). * p < 0.05

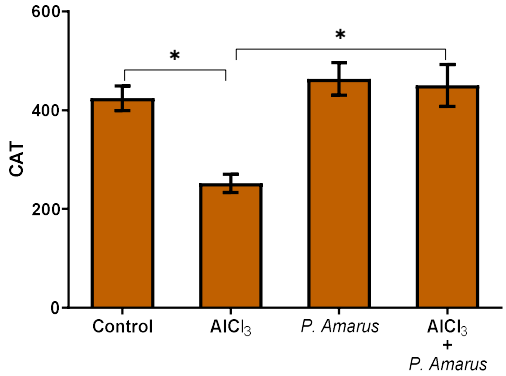

Fig. 5: Effect of $AlCl_3$ and *P. amarus* on CAT activity. Data is presented as mean ± SEM (n = 5, 50 drosophilas/vial). * p < 0.05

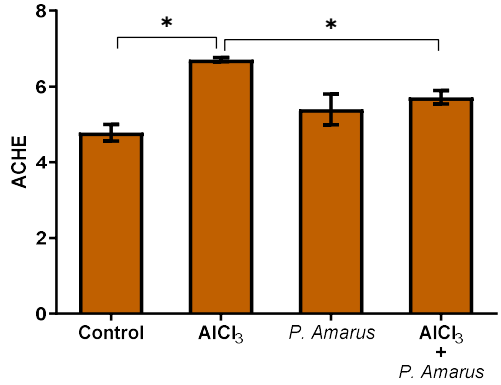

Fig. 6: Effect of $AlCl_3$ and *P. amarus* on AChE activity. Data is presented as mean ± SEM (n = 5, 50 drosophilas/vial). * p < 0.05

DISCUSSION

Oxidative stress and impaired cholinergic transmission are documented mechanisms by which aluminium induces neurotoxicity and cognitive dysfunction (Maya et al. 2016). This study was designed to investigate the anticholinesterase and antioxidant potential of *P. amarus* on $AlCl_3$-induced toxicity in *Drosophila melanogaster*.

In this study, the effect of $AlCl_3$ and a possible protective effect of *P. amarus* on the survival of drosophilas were investigated. It was observed that $AlCl_3$ treatment significantly reduced the survival rate of drosophilas, thus confirming the harmful and toxic effect of aluminium on the lifespan of experimental drosophilas. This negative impact of $AlCl_3$ on the survival rate of drosophilas has also been previously reported (Kijak et al. 2014; Oboh et al. 2021). The decrease in the survival rate induced by $AlCl_3$ in treated drosophilas has previously been attributed to its impairment of the cholinergic system and an increase in oxidative stress (Oboh et al. 2021). The increase in oxidative stress in $AlCl_3$-treated drosophilas (as suggested by an increase in MDA concentration) in this study corresponds to an increased susceptibility of the experimental drosophilas to oxidative damage and a higher mortality rate. However, *P. amarus* was able to improve the survival rate of drosophilas, thus demonstrating its potent neuroprotective activity against $AlCl_3$-induced toxicity.

Negative geotaxis (climbing activity) is a marker of neurodegeneration and has also been used in drosophila to access neuromuscular functions (Oyetayo et al. 2020). In this study, we observed a reduction in climbing activity in $AlCl_3$-assaulted drosophilas, which further confirms the neurotoxicity of aluminium in impairing locomotion. Acetylcholine has been reported to play an important role in locomotion and motor function (Nascimento et al. 2019). The reduction in locomotion observed in $AlCl_3$-assaulted drosophilas in this study can be attributed to an increase in AChE activity in the $AlCl_3$-assaulted drosophilas. This finding agrees with previous studies demonstrating that impaired locomotion is linked to $AlCl_3$ treatment (Oyetayo et al. 2020; Inneh and Eiya 2023). *P. amarus* was observed to significantly improve locomotor performance and climbing activity in $AlCl_3$-treated drosophilas, thus corroborating with a previous study (Adedayo et al. 2022).

Oxidative stress has been implicated in the pathogenesis of AD (Simunkova et al. 2019) and is also a well-documented mechanism by which aluminium induces neurotoxicity and AD. MDA, an indicator of oxidative stress and marker of lipid peroxidation (Ayala et al. 2014), was evaluated in the experimental drosophilas. It was observed that $AlCl_3$ treatment significantly increased MDA concentration in drosophilas, thus confirming previous reports (Oboh et al. 2021; Adedayo et al. 2022). The increase in MDA concentration by $AlCl_3$ further confirms aluminium's ability to induce oxidative stress, which causes deleterious effects in tissues. Aluminium has been reported to stimulate an increased production of reactive oxygen species, which results in the induction of lipid peroxidation (Abdalla et al. 2019). However, following *P. amarus* co-treatment, *P. amarus* was able to attenuate the increase in MDA concentration observed in $AlCl_3$-treated drosophilas only. This demonstrates the ability of *P. amarus* to protect against the oxidative stress induced by aluminium, possibly due to its antioxidant nature and ability to scavenge free radical species, which in turn mitigates lipid peroxidation.

Accumulating evidence indicates that antioxidant therapy is a successful treatment strategy for AD (Collins et al. 2022). Antioxidants protect cells from oxidative damage by neutralising and reducing the insults of excess amounts of ROS (Enogieru and Momodu 2021). To investigate the antioxidant potential of *P. amarus*, the activity of SOD and CAT was evaluated. SOD and CAT activity were significantly inhibited in $AlCl_3$-assaulted drosophilas. This reduction in SOD and CAT can be attributed to the pro-oxidant effect of $AlCl_3$, leading to increased production of ROS (Bayliak et al. 2019). SOD catalyses the dismutation of the superoxide anion to H_2O_2, while CAT in turn catalyses the conversion of H_2O_2 into oxygen and water, thus reducing the risk associated with oxidative stress-mediated damage. The depletion of these endogenous antioxidants by $AlCl_3$ increased the susceptibility of drosophila to oxidative stress and damage. In drosophilas co-treated with $AlCl_3$ and *P. amarus*, SOD and CAT activities were observed to be significantly improved in experimental drosophilas. The increase in these endogenous antioxidants is suggestive that *P. amarus* possesses *in vivo* anti-oxidant properties, which can be attributed to the presence of flavonoids. Flavonoids are reported to be potent ROS scavengers and metal chelators (Rodríguez-Arce and Saldías 2021).

Inhibitors of AChE play an important role in augmenting acetylcholine and enhancing cholinergic neurotransmission in the brain. They also play a key role in reducing the aggregation of β-amyloid peptides in AD (Liu et al. 2022). To investigate the AChE potential of *P. amarus*, the activity of the enzyme AChE among treatment groups was investigated. A significant increase in AChE activity in $AlCl_3$-assaulted drosophilas was observed when compared with that of the control. This is in line with previous studies that reported an increase in AChE activity in $AlCl_3$-treated drosophilas (Ogunsuyi et al. 2021; Adedayo et al. 2022; Inneh and Eiya 2023) and rats (Wei et al. 2017). Interestingly, in this study, drosophilas co-treated with $AlCl_3$ and *P. amarus* demonstrated a reduction in AChE activity when compared with drosophilas treated with $AlCl_3$ alone. This finding suggests that *P. amarus* possesses AChE activity. Since AChE inhibition is currently one of the most effective therapeutic strategies for the treatment of AD, *P. amarus* could potentially be of therapeutic benefit in the management of AD. Overall, the protective activity of *P. amarus* against $AlCl_3$ toxicity is possibly mediated through its potent anticholinesterase and antioxidant effects.

Conclusion

This study has demonstrated that *P. amarus* possesses AChE-inhibiting and antioxidant potential and may be useful in enhancing cholinergic transmission and improving antioxidant status. These are vital properties needed in the development of novel therapeutic agents for the treatment and/or management of $AlCl_3$ toxicity and its related disorders.

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Conflict of Interest
None declared.

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Authors' Contribution
CAI designed and performed the experiments. CAI and ABE both analysed, interpreted the data, and prepared the draft manuscript. Both authors reviewed and approved the final draft of the manuscript.

REFERENCES

Abdalla, R.P., Kida, B.M.S., Pinheiro, J.P.S., Oliveira, L.F., Martinez, C.B.F. and Moreira, R.G. (2019) Exposure to aluminum, aluminum+manganese and acid pH triggers different antioxidant responses in gills and liver of Astyanax altiparanae (Teleostei: Characiformes: Characidae) males. Comparative Biochemistry and Physiology Part C: Toxicol Pharmacol. 215:33-40.

Abolaji, A.O., Adedara, A.O., Adie, M.A., Vicente-Crespo, M. and Farombi, E.O. (2018) Resveratrol prolongs lifespan and improves 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine -induced oxidative damage and behavioural deficits in Drosophila melanogaster. Biochem Biophys Res Commun. 503(2):1042–1048.

Abolaji, A.O., Kamdem, P.J., Lugokenski, H.T., Nascimento, K.T., Waczuk, P.E., Farombi, E.O., et al. (2014) Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in Drosophila melanogaster. Free Radic Biol Med. 71:99–108.

Adedapo, A., Ofuegbe, S., and Oguntibeju, O. (2014) The antidiabetic activities of aqueous leaf extract of phyllantus amarus in some laboratory animals. Intech. 1:1-24.

Adedayo, B.C., Ogunsuyi, O.B., Akinniyi, S.T. and Oboh, G. (2022) Effect of Andrographis paniculata and Phyllanthus amarus leaf extracts on selected biochemical indices in Drosophila melanogaster model of neurotoxicity. Drug Chem Toxicol. 45(1):407–416.

Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105: 121–126.

Aparupa, B.M.G., Banerjee, A. and Chattopadhyay, S. (2022) An insight into the potent medicinal plant Phyllanthus amarus Schum. and Thonn. Nucleus. 65(3):437-472.

Ayala, A., Muñoz, M.F. and Argüelles, S. (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014:360438.

Bayliak, M.M., Lylyk, M.P., Gospodaryov, D.V., Kotsyubynsky, V.O., Butenko, N.V., et al. (2019) Protective effects of alpha-ketoglutarate against aluminum toxicity in Drosophila melanogaster. Comp Biochem Physiol. Part - C: Toxicol Pharmacol. 217:41–53.

Collins, A.E., Saleh, T.M. and Kalisch, B.E. (2022) Naturally occurring antioxidant therapy in Alzheimer's disease. Antioxidants. 11(2):213.

Egbon, E.E., Olayioye, E.Y., Olayioye, S.A., Adesuyi, A.T. and Owolabi, T. (2017) Phytochemical screening and proximate composition of Phyllanthus amarus. Int Res J Plant Sci. 8(1):9-12.

Ellman, G.L., Courtney, K.D., Andres, V. and Feathers-Stone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88–95.

Enogieru, A.B. and Momodu, O.I. (2021) The developing cerebellum as a target for toxic substances: protective role of antioxidants. Cerebellum. 20:614–630.

Enogieru, A.B. and Omoruyi, S.I. (2022) Exploration of aqueous Phyllanthus amarus leaf extract as a protective agent in mercury chloride-exposed Wistar rats: A neurobehavioural study. J Appl Sci Environ Manag. 26(4):629-637.

Haam, J. and Yakel, J.L. (2017) Cholinergic modulation of the hippocampal region and memory function. J Neurochem. 142:111-121.

Inneh, C.A. and Eiya, B.O. (2023) Anticholinesterase activity and antioxidant effect of vitamin E in aluminium chloride induced toxicity in Drosophila melanogaster. J Afr Assoc Physiol Sci. 11(1):17-28.

Kijak, E., Rosato, E., Knapczyk, K. and Pyza, E. (2014) Drosophila melanogaster as a model system of aluminum toxicity and aging. J Insect Sci. 21:189–202.

Kostyuk, V.A. and Potapovich, A.I. (1989) Superoxide--driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. Biochem Int. 19(5):1117–1124.

Liu, Y., Uras, G., Onuwaje, I., Li, W., Yao, H., Xu, S., et al. (2022) Novel inhibitors of AChE and Aβ aggregation with neuroprotective properties as lead compounds for the treatment of Alzheimer's disease. Eur J Med Chem. 235:114305.

Maurer, S.V. and Williams, C.L. (2017) The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. Front Immunol. 8:1489.

Maya, S., Prakash, T., Madhu, K.D. and Goli, D. (2016) Multifaceted effects of aluminium in neurodegenerative diseases: A review. Biomed Pharmacother. 83:746–754.

Moss, D.E. (2020) Improving anti-neurodegenerative benefits of acetylcholinesterase inhibitors in Alzheimer's disease: Are irreversible inhibitors the future? Int J Mol Sci. 21(10):3438.

Nascimento, F., Spindler, L.R. and Miles, G.B. (2019) Balanced cholinergic modulation of spinal locomotor circuits via M2 and M3 muscarinic receptors. Sci Rep. 9(1):14051.

Oboh, G., Oladun, F.L., Ademosun, A.O. and Ogunsuyi, O.B. (2021) Anticholinesterase activity and antioxidant properties of Heinsia crinita and Pterocarpus soyauxii in Drosophila melanogaster model. J Ayurveda Integr Med. 12(2):254-260.

Ogunsuyi, O.B., Oboh, G., Özek, G. and Göger, F. (2021) Solanum vegetable-based diets improve impairments in

memory, redox imbalance, and altered critical enzyme activities in Drosophila melanogaster model of neurodegeneration. J Food Biochem. 45(3):e13150.
Ohbe, H., Jo, T., Matsui, H., Fushimi, K. and Yasunaga, H. (2018) Cholinergic crisis caused by cholinesterase inhibitors: a retrospective nationwide database study. J Med Toxicol. 14(3):237-241.
Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95(2):351–358.
Oyetayo, B.O., Abolaji, A.O., Fasae, K.D. and Aderibigbe, A. (2020) Ameliorative role of diets fortified with Curcumin in a Drosophila melanogaster model of aluminum chloride-induced neurotoxicity. Biomed Pharmacother. 71:104035.
Rodríguez-Arce, E. and Saldías, M. (2021) Antioxidant properties of flavonoid metal complexes and their potential inclusion in the development of novel strategies for the treatment against neurodegenerative diseases. Arch Toxicol. 143:112236.
Santos, T.C.D., Gomes, T.M., Pinto, B.A.S., Camara, A.L. and Paes, A.M. (2018) Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. Front Pharmacol. 9:1192.
Shunan, D., Yu, M., Guan, H. and Zhou, Y. (2021) Neuroprotective effect of betalain against $AlCl_3$-induced Alzheimer's disease in Sprague Dawley rats via putative modulation of oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway. Biomed Pharmacother. 137:111369.
Simunkova, M., Alwasel, S.H., Alhazza, I.M., Jomova, K., Kollar, V., Rusko, M., et al. (2019) Management of oxidative stress and other pathologies in Alzheimer's disease. Arch Toxicol. 93:2491-2513.
Wang, Z., Wei, X., Yang, J., Suo, J., Chen, J., Liu, X., et al. (2016) Chronic exposure to aluminum and risk of Alzheimer's disease: A meta-analysis. Neurosci Lett. 610: 200-206.
Wei, Y., Liu, D., Zheng, Y., Li, H., Hao, C. and Ouyang, W. (2017) Protective effects of kinetin against aluminum chloride and D-galactose induced cognitive impairment and oxidative damage in mouseBrain Res. Bull. 134: 262–272.
Wu, Z., Du, Y., Xue, H., Wu, Y. and Zhou, B. (2012) Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production. Neurobiol Aging. 33(1): 1-12.