



Antioxidant and anticholinesterase activity of rutin in aluminum chloride-exposed *Drosophila melanogaster*

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Abstract

Aluminum (Al) toxicity has been associated with multiple neurodegenerative disorders, including Alzheimer's disease (AD). Since current medications for AD and Al-related toxicity are reported to have significant side effects, the search for therapeutic agents possessing antioxidant and anticholinesterase potential is ongoing. One such agent is rutin, a flavonoid, reported for its potent antioxidant and anti-inflammatory effects. This study investigated the activity of rutin in mitigating Al chloride (AlCl₃) toxicity in *Drosophila melanogaster*. Flies were divided into six groups containing fifty (50) flies each. Group A served as the control; Group B received 40 mM AlCl₃; Groups C and D were co-treated with 40 mM AlCl₃ + 0.5 mg/kg rutin and 40 mM AlCl₃ + 1 mg/kg, respectively; Groups E and F were treated with rutin alone in doses of 0.5 mg/kg and 1 mg/kg, respectively, all through their diet. Negative geotaxis, superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and acetylcholinesterase (AChE) activities were evaluated at the end of the study. A marked decrease ($P < 0.05$) was noticed in survival rate, negative geotaxis, and SOD as well as a significant increase in MDA and AChE in the AlCl₃-exposed flies when compared to the control. Conversely, in the groups co-treated with rutin, there was a significant attenuation of the negative effects of AlCl₃. Taken together, these findings suggest that rutin protected against AlCl₃, thus indicating the possible therapeutic effects of rutin against aluminum toxicity and its related disorders.

Keywords Rutin · *Drosophila melanogaster* · Aluminum · Neurotoxicity

Introduction

Aluminum (Al) ranks as the third most prevalent element in the Earth's crust and is ubiquitous in our surroundings, encompassing the foods we consume (Klein 2019). Al is commonly accessible to both animal and human populations, making intoxication a potential concern. Aluminum can find its way into the body through various means, such as inhaling aerosols or particles, skin contact, vaccination, dialysis, infusions, and ingesting contaminated food, water, or medications. The adverse effects of aluminum toxicity encompass a wide range of issues, including oxidative stress, alterations in the immune system, genetic damage, inflammation, modification of peptides, enzyme

dysfunction, disruptions in metabolic processes and iron homeostasis, amyloid formation, and disturbance of cell membranes, as well as cellular responses like apoptosis, necrosis, and abnormal cell growth (Igbokwe et al. 2019). Some of these cases are linked to the fact that Al is a neurotoxic substance, which has been discovered in elevated levels within the brain tissues of individuals with conditions such as Alzheimer's disease (AD), epilepsy, and autism (Alasfar and Isaifan 2021). Reports indicate that Al exposure inhibits acetylcholine in the brain, thus establishing a link between the potential involvement of Al in the development of AD (Dave et al. 2002). Acetylcholinesterase (AChE), a serine hydrolase enzyme, plays a pivotal role in breaking down the neurotransmitter acetylcholine. Consequently, inhibiting AChE activity has emerged as a promising approach for AD treatment.

Various natural products derived from diverse plant sources are increasingly gaining global recognition as potential AChE inhibitors and offering a potential therapeutic avenue for addressing AD (Taqui et al. 2022). Due to the lack of effective remedies to prevent, reverse,

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or halt neuronal loss, researchers have turned to plant-derived bioactive compounds as potential treatments for these debilitating conditions; one of such bioactive compounds of interest is rutin. Rutin, a glycoside of the flavonoid quercetin naturally found in various plants and fruits like grapes, apricots, buckwheat, plums, cherries, and oranges, has shown promising effects in various disease conditions and has generated significant attention for its therapeutic potential in different models of neurodegenerative diseases (Enogieru et al. 2018). It possesses antiallergic, anti-inflammatory, antimicrobial, anticancer, and neuroprotective effects. Additionally, rutin exhibits notable antioxidant effects effectively scavenging 1,1-diphenyl radical-2-picrylhydrazyl (DPPH) and efficiently inhibiting lipid peroxidation in experimental studies. Its cytoprotective properties have also been demonstrated in studies involving invertebrate models. Rutin's remarkable attributes make it a valuable compound in the ongoing quest for new therapeutic substances needed to treat various neurodegenerative disorders (Fidelis et al. 2021).

Drosophila melanogaster, also known as fruit fly or vinegar fly, is an arthropod belonging to the Drosophilae family. Over 100 years ago, William Castle introduced this dipteran insect as a model organism for biological research. It has been extensively employed in studying human genetics, mammalian physiology, microbial pathogenesis, life history evolution, genotoxicity, and cancer. One of the reasons for its widespread use is the significant similarities it shares with humans, making it a valuable tool for studying complex cellular signaling pathways that regulate development and survival (Jennings 2011). Approximately 75% of genes responsible for human diseases have comparable functional homologues in *Drosophila melanogaster*. This insect species is highly cost-effective to maintain in the laboratory and is recommended as a valuable alternative model for studying diseases, thus surpassing the need for vertebrate models (Abolaji et al. 2013). Accordingly, this study investigated the anticholinesterase and antioxidant potential of rutin against aluminum chloride-induced toxicity in *Drosophila melanogaster*.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Aluminum chloride (CAS no. 7784-13-6) manufactured by GHTECH (China) was purchased from Pyrex Chemicals in Benin City, Edo State. Additional chemicals utilized such as acetylthiocholine iodide and rutin were purchased from Sigma-Aldrich (St. Louis, MO).

Study subject

Drosophila melanogaster (Harwich strain) of both genders of 3–5 days old were obtained from the *Drosophila* laboratory of the University of Ibadan, Oyo state, Nigeria. The flies were reared on a cornmeal-based diet which contained 1% w/v agar, 1% w/v brewer's yeast, 1% w/v powdered milk, 2% w/v glucose, and 0.08% v/w nipagin at room temperature (24 °C) under a 12-h dark/light cycle condition at the *Drosophila* laboratory of Central Research Laboratory, University of Benin, Benin City. The same strain of *Drosophila melanogaster* was utilized during the experiment.

Experimental design

A controlled experiment was conducted with five (5) treatment groups and one (1) control group to evaluate the effect of the treatment across experimental groups. Group A flies were placed on a cornmeal diet and distilled water, while Group B flies were placed on a cornmeal diet containing AlCl₃ alone; Groups C and D were placed on a cornmeal diet containing rutin (0.5 and 1 mg/kg) and AlCl₃, respectively; Groups E and F were placed on a cornmeal diet containing rutin alone (0.5 and 1 mg/kg), as described in Table 1.

Survival study

Following appropriate treatment according to the experimental design, the flies were observed daily for mortality and the survival rate was determined by counting the number of dead flies during the 14-day treatment (Abolaji et al. 2017). The data was subsequently analyzed and plotted as a percentage of survival after the treatment period (Akinsanmi et al. 2019; Igharo et al. 2021). The survival assay was carried out in three replicates of each concentration.

Negative geotaxis assay

This assay was used to determine the locomotor performance or climbing activity of the flies. This assay was carried out as previously described (Abolaji et al. 2018). Ten (10) flies

Table 1 Experimental design

Groups	Dosage
Group A	Control (distilled water)
Group B (AlCl ₃)	40 Mm AlCl ₃
Group C (RUT1 + AlCl ₃)	0.5 mg/kg of rutin + 40 Mm AlCl ₃
Group D (RUT2 + AlCl ₃)	1 mg/kg of rutin + 40 Mm AlCl ₃
Group E (RUT1)	0.5 mg/kg of rutin
Group F (RUT2)	1 mg/kg of rutin

from each group were immobilized under ice anesthesia. They were subsequently placed separately in labeled 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 flies were allowed to climb. The number of flies that climbed up to and above the 6 cm mark of the column in 6 s as well as those that remained below this mark after this time was recorded. The climbing activity was scored by expressing the proportion (%) of flies above the 6 cm mark. After 1 min interval, this procedure was repeated. A total of three repetitions were carried out for each group.

Preparation of samples for biochemical assays

At the end of a 7-day treatment period, the flies (intact whole body) were homogenized and centrifuged, and the supernatants were used for biochemical analysis. The flies were first anesthetized in ice, thereafter weighed, and then homogenized in 0.1 M potassium phosphate buffer of pH 7.4 (1:10) (Oyetayo et al. 2020). They were later centrifuged at 4000 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), and acetylcholinesterase (AChE) activities.

Superoxide dismutase (SOD) activity

SOD activity was determined as previously described (Misra and Fridovich 1972). The reaction mixture contained 10 μ L of the sample, 15% quercetin, 20 mM phosphate buffer (pH 7.8), 0.08 mM EDTA, and 8 mM tetramethylethylenediamine (TEMED). The reaction was left for 3 min, and absorbance was measured at 406 nm. The results were expressed as the amount of protein required to inhibit

quercetin auto-oxidation (μ mol/min/mg protein) (Misra and Fridovich 1972).

Determination of catalase activity

Catalase activity was determined following previous methods (Cohen et al. 1970). Ten μ L of the sample (1:50 dilution) and 50 mM potassium phosphate buffer (pH 7.0) followed by 300 mM H_2O_2 were mixed. The loss in absorbance of H_2O_2 was monitored for 2 min at 240 nm and was subsequently used to calculate catalase activity which was expressed as μ mol of H_2O_2 consumed per minute per milligram of protein (Cohen et al. 1970).

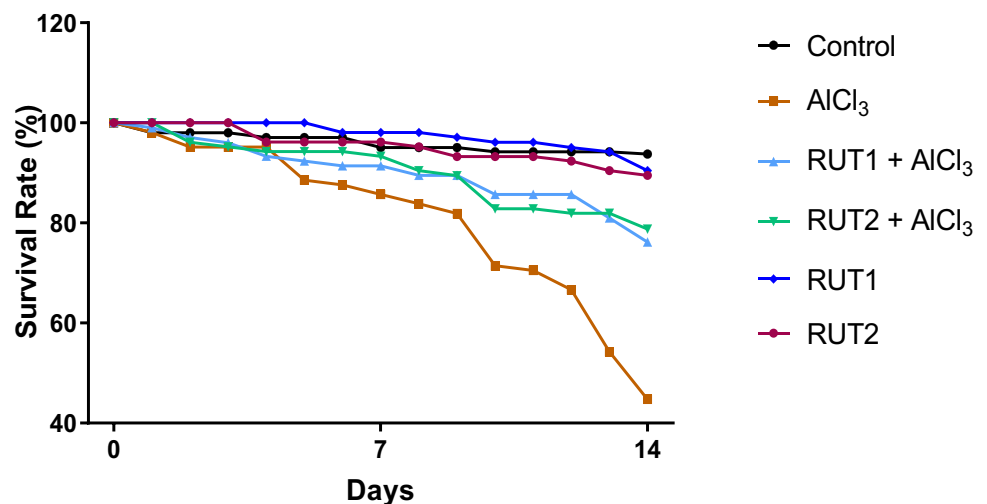
Determination of lipid peroxidation

Lipid peroxidation was determined according to a previous method (Varshney and Kale 1990). The mixture contained 40 μ L of the supernatant, 100 μ L of 0.67% thiobarbituric acid, 5 μ L of 10 mM butyl-hydroxytoluene (BHT), 300 μ L of 1% O-phosphoric acid, and 55 μ L 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 μ mol MDA formed per milligram protein (Varshney and Kale 1990).

Determination of acetylcholinesterase activity

Acetylcholinesterase activity was evaluated following the method previously described (Ellman et al. 1961). The reaction mixture contained 30 μ L 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 μ mol of acetylthiocholine hydrolyzed per min per milligram protein (Ellman et al. 1961).

Fig. 1 Effect of $AlCl_3$ and rutin on the survival rate of flies (days 0–14)



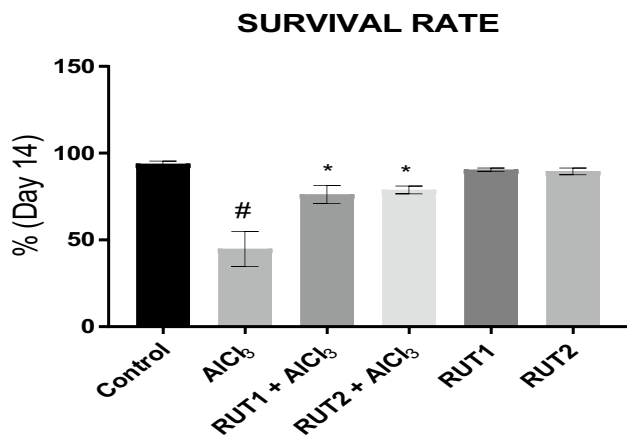


Fig. 2 Effect of AICl₃ and rutin on the survival rate of flies (day 14). # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AICl₃ group

Statistical analysis

Data was analyzed using the GraphPad Prism statistical package (version 7). Statistical significance ($P < 0.05$) was determined through analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. Results are presented as mean \pm standard error of mean (mean \pm SEM).

Results

Survival rate of *D. melanogaster* exposed to AICl₃ and rutin

Figures 1 and 2 illustrate the survival rate in experimental Groups A–F. Figure 1 shows the survival rate of flies from days 0 to 14. On the 14th day (Fig. 2), there was a significant decrease ($P < 0.05$) for Group B (44.76%) when compared to the control (93.76%), while there was a significant increase ($P < 0.05$) in Group C (RUT1 + AICl₃; 76.19%) and Group D (RUT2 + AICl₃; 78.76%) when compared to Group B (AICl₃). However, there were no significant differences ($P > 0.05$) in survival rates between Groups E (RUT1; 90.47%) and F (RUT2; 89.52%) when compared to the control group.

Climbing activity (negative geotaxis) of *D. melanogaster* exposed to AICl₃ and rutin

Figure 3 illustrates the climbing activity in experimental Groups A–F. There was a significant decrease ($P < 0.05$) in the climbing activity of Group B when compared to the control, while there was a significant increase ($P < 0.05$) in Groups C (RUT1 + AICl₃) and D (RUT2 + AICl₃) when compared to Group B (AICl₃). Nevertheless, there were

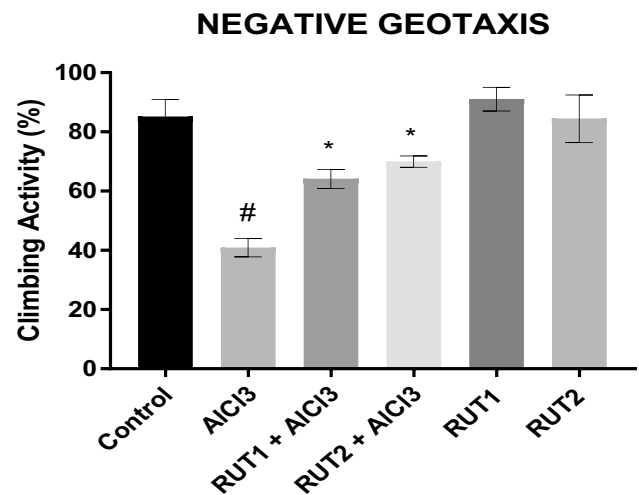


Fig. 3 Effect of AICl₃ and rutin on climbing activity (negative geotaxis). # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AICl₃ group

no significant differences ($P > 0.05$) in climbing activity between Groups E (RUT1) and F (RUT2) when compared to the control group.

Superoxide dismutase (SOD) activity of *D. melanogaster* treated with AICl₃ and rutin

Figure 4 illustrates the superoxide dismutase (SOD) activity in experimental Groups A–F. A significant decrease ($P < 0.05$) was noted in Group B when compared to the control. Conversely, there was a significant increase ($P < 0.05$) in SOD activity in both Groups C (RUT1 + AICl₃) and D (RUT2 + AICl₃) when compared to Group B (AICl₃). However, there were no significant differences ($P > 0.05$) in SOD activity between Groups E (RUT1) and F (RUT2) when compared to the control group.

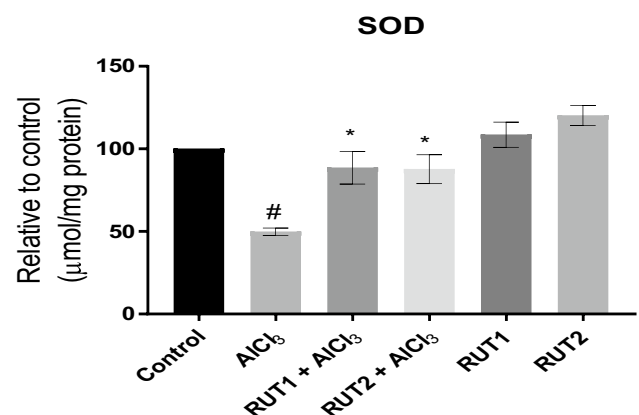


Fig. 4 Effect of AICl₃ and rutin on superoxide dismutase (SOD) activity. # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AICl₃ group

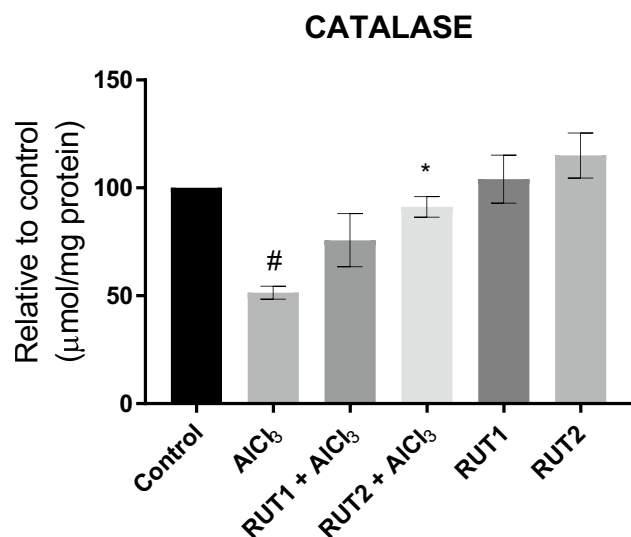


Fig. 5 Effect of AlCl₃ and rutin on catalase (CAT) activity. # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AlCl₃ group

Catalase (CAT) activity of *D. melanogaster* treated with AlCl₃ and rutin

Figure 5 illustrates the catalase (CAT) activity in experimental Groups A–F. A significant decrease ($P < 0.05$) was noted in Group B when compared to the control. Conversely, there was a significant increase ($P < 0.05$) in CAT activity in Group D (RUT2 + AlCl₃) when compared to Group B (AlCl₃). However, there were no significant differences ($P > 0.05$) in CAT activity between Groups E (RUT1) and F (RUT2) when compared to the control group.

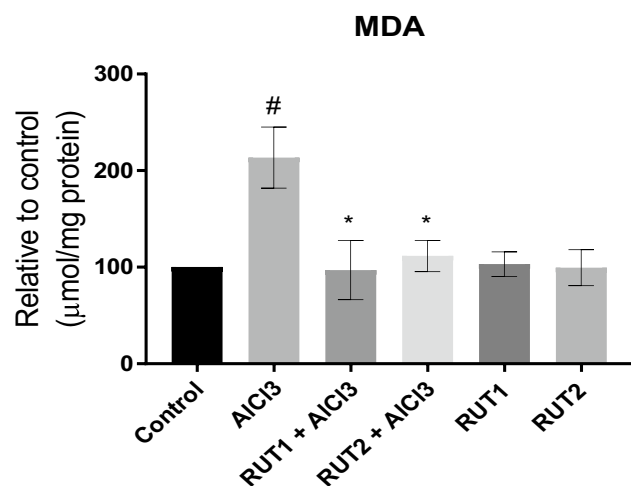


Fig. 6 Effect of AlCl₃ and rutin on lipid peroxidation (MDA) activity. # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AlCl₃ group

Lipid peroxidation (MDA) activity of *D. melanogaster* treated with AlCl₃ and rutin

Figure 6 illustrates the lipid peroxidation (MDA) activity in experimental Groups A–F. A significant increase ($P < 0.05$) was noted in Group B when compared to the control. Conversely, there was a significant decrease ($P < 0.05$) in MDA activity in both Groups C (RUT1 + AlCl₃) and D (RUT2 + AlCl₃) when compared to Group B (AlCl₃). However, there were no significant differences ($P > 0.05$) in MDA activity between Groups E (RUT1) and F (RUT2) when compared to the control group.

Acetylcholinesterase (AChE) activity of *D. melanogaster* treated with AlCl₃ and rutin

Figure 7 illustrates the AChE activity in experimental Groups A–F. A significant increase ($P < 0.05$) was noted in Group B (AlCl₃) when compared to the control. In contrast, both Groups C (RUT1 + AlCl₃) and D (RUT1 + AlCl₃) displayed a significant decrease ($P < 0.05$) in AChE activity compared to Group B (AlCl₃). No significant differences ($P > 0.05$) in AChE activity were evident in Groups E (RUT1) and F (RUT2) when compared to the control group.

Discussion

The fruit fly (*Drosophila melanogaster*) has been firmly established as a model organism for the study of neurotoxicity and neurodegenerative diseases (Adedayo et al. 2022). Its extensive genetic toolbox allows for precise and highly targeted genome manipulations, leading to significant breakthroughs. Additionally, its relatively short lifespan enables researchers to investigate intricate aspects of brain function more rapidly compared to other model organisms like mice

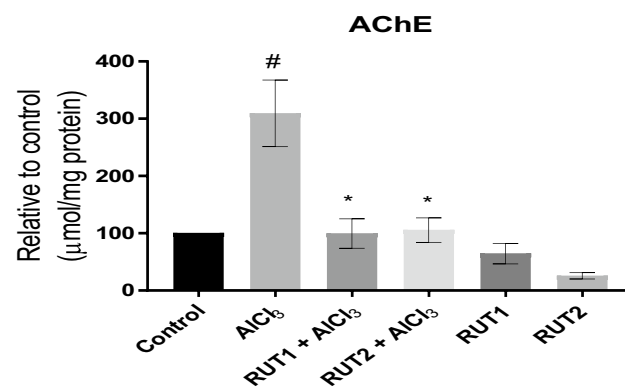


Fig. 7 Effect of AlCl₃ and rutin on acetylcholinesterase (AChE) activity. # $P < 0.05$ compared with the control group; * $P < 0.05$ compared with the AlCl₃ group

(McGurk et al. 2015). The survival rate in *Drosophila* refers to the proportion of flies that remain alive or survive within a given population or group over a specified period, often in the context of experiments or studies involving the effects of various factors like toxic substances, diets, or treatments on the fly population's longevity (Akinsanmi et al. 2019; Farombi et al. 2018). The substantial decline in the survival rate of *Drosophila melanogaster* subjected to AlCl_3 exposure alone serves as clear evidence of the detrimental effects of aluminum chloride; this is consistent with other studies demonstrating that AlCl_3 significantly reduces the survival rate of *Drosophila melanogaster* via oxidative stress (Oyetayo et al. 2020; Oboh et al. 2021).

Negative geotaxis, a natural reflexive behavior in *Drosophila*, involves the flies climbing upwards along the inner wall of a cylindrical container in response to a stimulus, for instance, a gentle tap at the bottom (Rhondenizer et al. 2008). Investigations confirmed that negative geotaxis in *Drosophila* represents an age-related decline in climbing primarily due to reduced climbing speed. This decrease in locomotor speed with age is a characteristic feature of declining locomotor function observed in both flies and humans (Gargano et al. 2005; Rhondenizer et al. 2008). For this study, the notable decrease observed in the climbing activity of *Drosophila melanogaster* exposed to aluminum chloride alone underscores its harmful impact; this aligns with the findings reported previously (Inneh and Eiya 2023; Oyetayo et al. 2020).

Organisms have a defense mechanism to safeguard their tissues by secreting enzymes possessing antioxidant properties which serve to neutralize or eliminate damaging oxygen species (Beyer et al. 1991). SOD regulates oxidative stress, lipid metabolism, inflammation, and oxidation, preventing lipid peroxidation, low-density lipoprotein oxidation, and inflammatory cell adhesion. As a primary antioxidant enzyme, SOD converts superoxide radicals into hydrogen peroxide and oxygen, safeguarding tissues from harmful oxygen species (Beyer et al. 1991; Islam et al. 2022). The findings from this study demonstrate a significant reduction in superoxide dismutase (SOD) levels in flies exposed solely to AlCl_3 when compared to the control group. This suggests that aluminum has the potential to disrupt antioxidant functions by inhibiting antioxidant enzymes; this is consistent with other studies (Efosa et al. 2023; Inneh and Enogieru 2023). Rutin exhibited the capacity to augment SOD activity when administered concurrently, implying its potential to enhance the antioxidant properties of SOD; this is consistent with other studies which showed rutin increased SOD activity in rodent models (Arowoogun et al. 2021; Prasad and Prasad 2019; Qu et al. 2019). Catalase (CAT), known as hydrogen peroxide/hydrogen peroxide oxidoreductase, stands as a vital intracellular antioxidant enzyme, safeguarding cells against oxidative stress, located

within the peroxisomes of aerobic cells (Shangari and O'Brien 2006). Its primary role involves shielding the cell from the harmful impacts of elevated hydrogen peroxide (H_2O_2) levels. This is achieved by catalyzing the breakdown of H_2O_2 into molecular oxygen and water, without generating free radicals in the process (Shangari and O'Brien 2006). The findings from this study demonstrate a reduction in CAT levels in flies exposed solely to AlCl_3 when compared to the control group. This suggests that aluminum has the potential to disrupt antioxidant functions by inhibiting the activity of CAT; this is consistent with other studies (Ahmad Rather et al. 2019; Inneh and Enogieru 2023; Liaquat et al. 2019). Rutin exhibited the capacity to augment CAT activity when administered concurrently, implying its potential to enhance the antioxidant properties of CAT; this is consistent with other studies which showed rutin increased CAT activity in rodent models (Iova et al. 2021; Uthra et al. 2022).

Lipid peroxidation is an important mechanism in free radical-mediated cell injury. It has the potential to directly harm cellular membranes, and the resulting reactive carbonyl byproducts could extend the damage well beyond the initial location of radical generation. It has long been considered to be involved in various toxic tissue injuries and certain disease processes (Cheeseman 1993). Lipid peroxidation plays a significant role in crucial cellular antioxidant mechanisms. The processes by which iron, oxygen, and specific hepatotoxins induce tissue damage often revolve around the creation of intermediate free radical species and the initiation of lipid peroxidation (Protchenko et al. 2021). Malondialdehyde (MDA) serves as a conclusive byproduct resulting from the peroxidation of polyunsaturated fatty acids within cellular systems. Elevated levels of free radicals contribute to an excessive production of MDA. The quantification of MDA levels is widely recognized as a pivotal indicator of oxidative stress and antioxidant status (Gawel et al. 2004). In this study, the substantial elevation in MDA when exposed to AlCl_3 is consistent with earlier studies (Akpanyung et al. 2020; Kumar et al. 2009). Rutin exhibited the capacity to reduce MDA activity when administered concurrently, implying its potential to decrease lipid peroxidation. This is consistent with other studies which showed rutin reduced MDA activity in rodent models (Korkmaz, and Kolankaya 2010; Rotimi et al. 2023).

Acetylcholinesterase (AChE) is recognized as a pivotal biomarker in AD. Inhibitors of AChE hold substantial promise for AD therapy due to AChE's role in augmenting the neurotoxicity associated with the amyloid component implicated in AD pathogenesis. Consequently, the development of a straightforward and exceptionally sensitive method for monitoring AChE levels and identifying potent AChE inhibitors is of utmost importance and significance (Wang et al. 2023). Acetylcholinesterase plays a pivotal role in terminating impulse transmission by swiftly breaking down

the neurotransmitter acetylcholine in multiple cholinergic pathways within the central and peripheral nervous systems (Colović et al. 2013). In this study, we evaluated the effects of $AlCl_3$ on the activity of AChE; it was observed that the activity of AChE increased in flies subjected to aluminum exposure and agrees with other studies with fly models (Zatta et al. 2002; Oboh et al. 2021). On the other hand, rutin was able to lower the activity of this enzyme, which highlights its effectiveness as a promising acetylcholinesterase inhibitor and might hold therapeutic significance in the treatment and/or management of neurodegenerative disorders such as Alzheimer's disease (Xie et al. 2014; Oboh et al. 2020).

Conclusion

The results of this study suggest that rutin's protective activity against $AlCl_3$ toxicity is mediated through its potent antioxidant and anticholinesterase properties. Consequently, rutin may have potential applications in the treatment and/or management of neurological disorders associated with aluminum exposure.

Compliance with ethical standards

Funding This study was not supported by any funding.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Research Ethics Committee, College of Medical Sciences, University of Benin, with approval no. CMS/REC/2023/356.

Informed consent For this type of study, informed consent is not required.

Consent for publication For this type of study, consent for publication is not required.

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