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Toxicity and genotoxicity of $AlCl_3$ in transgenic *Drosophila melanogaster (hsp-70 lacZ) Bg*^{9}

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ABSTRACT

Drosophila melanogaster third-instar larvae were exposed to $AlCl_3$ in the diet for 24 h. Dose-dependent reductions in pupation and adult emergence were observed, Biochemical analyses revealed significantly elevated oxidative stress markers: increased lipid peroxidation and protein oxidation and depletion of reduced glutathione and antioxidant enzymes such as superoxide dismutase, catalase, and glutathione transferase. At higher concentrations, larvae also showed increased β-galactosidase activity and ONPG hydrolysis, suggesting lysosomal dysfunction and senescence-like responses.

1. Introduction

Metals, many of which are vital for biological processes, occur naturally in the Earth's crust [1]. Aluminum (Al), the third most abundant element, has broad industrial applications, including in the pharmaceutical, chemical, construction, and food processing industries [2]. Under natural conditions, Al has low bioavailability; however, human activities have significantly increased its mobilization, turning it into a pervasive environmental toxicant [3]. Al is present in the human diet, both as a natural food component and as an additive in processed products. Processed cheese, bakery products, cereals, soft drinks, coffee, rice, biscuits, and dairy substitutes often contain Al additives [4]. Compounds such as sodium aluminum sulfate, sodium aluminum phosphate, and sodium aluminosilicate serve as stabilizers in food manufacturing. According to the World Health Organization (WHO), the tolerable daily intake is 1 mg Al per kg body weight (WHO, 2023). Nevertheless, current levels of exposure may exceed this limit. Al exposure arises from multiple sources, including diet, food additives, environment, industry effluents, and occupational settings [5–6]. Certain crops, including rice, potatoes, spinach, herbs, and spices, accumulate Al at higher levels [7–8]. Al concentration in fruits and vegetables is influenced by factors such as soil acidity, irrigation water quality, and plant type [9].

The extent of Al absorption depends strongly on the route of exposure, with ingestion of food and water being the primary source of systemic uptake. Gastrointestinal absorption has been documented in several invertebrate models, including *Drosophila* [10–11], the land snail *Helix aspersa* [12–13], the crayfish *Pacifastacus leniusculus* [14], the nematode *Caenorhabditis elegans* [15], and the earthworm *Eisenia andrei* [16]. Al toxicity arises through several mechanisms, including competition with essential metals, alteration of protein structures [17], and induction of oxidative stress [18]. Recent work in *Drosophila* and other model systems shows that $AlCl_3$ exposure causes oxidative stress and developmental and neurobehavioral deficits. These findings underscore the toxicity of Al and the value of *Drosophila* for mechanistic and mitigation studies [19–21].

Drosophila melanogaster is a useful model organism in toxicology studies [22–24]. Its suitability arises from the ease with which compounds can be incorporated into the diet of larvae or adults, enabling investigation of effects on development, morphology, and physiology and making *Drosophila* a practical system for assessing metal toxicity [25]. The fly's short life-cycle allows researchers to study developmental and adult-stage toxicity within a short time-frame. *Drosophila* shares significant genetic similarity with humans, with approximately 75 % of

Abbreviations: SOD, Superoxide dismutase; CAT, Catalase; GSH, Gluatathione; GST, Glutathione Transferase; PCC, Protein carbonyl content; LPO, lipid peroxidation; TBARS, thiobarbituric acid reactive species; MDA, malondialdehyde; ROS, Reactive Oxygen Species.

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human disease-related genes having orthologs in the fly genome [26]. This conservation enhances the relevance of findings to human health.

We have investigated the effects of $AlCl_3$ on third-instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ)* Bg^9.

2. Materials and methods

2.1. Fly strain and treatment

Drosophila melanogaster (hsp70-lacZ) Bg^9 transgenic strain, used in the present study, is genetically modified to produce bacterial β-galactosidase along with Hsp70 in response to stress [27]. $AlCl_3$ was purchased from SRL (India) and mixed in the diet at final concentrations of 20, 40, 60, and 80 μM. The concentrations of $AlCl_3$ were incorporated during the preparation of the diet. While the diet remained in a liquid state, an $AlCl_3$ stock solution was prepared and added in appropriate amounts to achieve final concentrations of 20, 40, 60, and 80 μM. After thorough mixing to ensure uniform distribution, the diet was allowed to solidify.

The LC_{50} value for $AlCl_3$, determined by exposing third-instar larvae to a range of $AlCl_3$ concentrations, was approximately 400 μM. The highest dose used in the present study was less than one-fourth of this LC_{50} value.

2.2. O-nitrophenyl-β-D-galactopyranoside (ONPG) assay

Hsp70 expression serves as a valuable indicator of cytotoxicity [28]. The method of Nazir et al. [29] was used. Larvae were placed in Eppendorf tubes (30 larvae per tube; five replicates per group) and rinsed with phosphate buffer. They were permeabilized with acetone and incubated in O- Nitrophenol beta galactopyraniside (ONPG) buffer consisting of $MgSO_4$, Na_2HPO_4, $Na_2H_2PO_4$, ONPG, beta-mercaptoethanol and KCl 600 μl.

The reaction was stopped by adding Na_2CO_3, (1 M) 300 μl.

The reaction was quantified by measuring absorbance at 420 nm.

2.3. In situ histochemical β-galactosidase activity

Ten larvae per treatment group were dissected in Pole's salt solution (PSS) according to the method of Chowdhuri et al. [28], with each group having five replicates. The tissue explants tissue samples were incubated overnight in X-gal staining solution. The samples were fixed in 2.5 % glutaraldehyde solution and subsequently washed with sodium phosphate buffer and observed under the microscope.

2.4. Trypan blue exclusion test

Tissue damage in the larvae was evaluated by the Trypan blue exclusion assay [30]. The explants of ten larvae per treatment (five replicates per group) were isolated and rinsed in phosphate-buffered saline (PBS). The larvae were stained with Trypan blue for 30 min, thoroughly washed in PBS, and assessed for dark blue staining. Scoring was performed using a composite index for each larva: no color = 0; any blue color = 1; dark blue staining = 2; large patches of dark staining = 3; and complete staining of most cells = 4 [31].

2.5. Comet assay

The comet assay was performed following the method of Mukhopadhyay et al. [32]. Base slides were prepared by heating 1 % normal-melting agarose (NMA), coating one-third of frosted slides, and allowing them to solidify at room temperature. Midguts were isolated from 20 larvae per treatment group, with three biological replicates per group, and collected in PBS, 50 μl. After removing the PBS, collagenase (0.5 mg/ml in PBS, pH 7.4, 300 μl) was added, and the samples were incubated before slide preparation. For embedding, 75 μl of the cell suspension was mixed with 1.5 % low-melting-point agarose (LMPA), 80 μl, applied onto the pre-coated slides, covered with a coverslip for 10 min, and then the coverslip was removed. For electrophoresis, slides were placed in the chamber and covered with a chilled alkaline solution (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) for a 10-min. unwinding period. Electrophoresis was then performed for 15 min at 0.7 V/cm and 300 mA, at 4 °C. Following electrophoresis, slides were rinsed three times with neutralizing buffer (0.4 M Tris) and stained in the dark for 10 min with ethidium bromide (20 μg/ml; 75 μl per slide). The slides were subsequently rinsed with chilled distilled water and covered with coverslips. Cells were analyzed under a fluorescence microscope (Optika, Italy). Using Comet Score 1.5 software, 25 cells per slide were randomly selected, and the mean tail length (in arbitrary units) was calculated to evaluate DNA damage.

2.6. Preparation of homogenate for biochemical assays

The larvae (100 larvae/treatment; five replicates/group) and control third instar larvae fed only on diet were homogenized in cold homogenizing buffer (0.1 M phosphate buffer containing 0.15 M KCl; pH 7.4, 1 ml). The supernatant obtained after centrifugation at 9000 × g was used for determination of glutathione (GSH) content, glutathione transferase (GST) activity, protein carbonyl content (PCC), and TBARS levels, using a spectrophotometer (Model: UV-1800 Shimadzu UV Spectrophotometer) for absorbance measurements.

2.6.1. Glutathione (GSH) content

Glutathione (GSH) content was estimated calorimetrically using Ellman's reagent according to the procedure of Jollow et al. [33]. The supernatant was precipitated with 4 % sulphosalicyclic acid in the ratio of 1:1. The samples were kept at 4 °C for 1 h and then subjected to centrifugation at 4200 rpm for 10 min. at 4 °C. The assay mixture consisted of 0.1 M phosphate buffer, 550 μl; supernatant, 100 μl; and 5, 5-dithio-bis-(2-nitrobenzoic acid) (DTNB) 100 μl (0.01 M). Absorbance was read at 412 nm and the results were expressed as micromol GSH/g tissue.

2.6.2. Glutathione transferase (GST) activity

Glutathione transferase (GST) activity was determined using the method of Habig *et al.* [34]. The reaction mixture consisted of 0.1 M phosphate buffer, 500 μl; 10 mM 1-chloro-2,4-dinitrobenzene (CDNB), 150 μl; 10 mM reduced glutathione, 200 μl; and supernatant, 50 μl. Absorbance was measured at 340 nm and enzyme activity was expressed as micromole CDNB conjugate/min/mg protein.

2.6.3. TBARS assay

TBARS were measured according to the method of Ohkawa *et al.* [35]. The reaction mixture consisted of 10 mM *tert*-butyl-hydroxytoluene (BHT), 5 μl; 0.67 % thiobarbituric acid, 200 μl; 1 % phosphoric acid, 600 μl; distilled water, 105 μl; and supernatant, 90 μl. The resulting mixture was incubated at 90 °C for 45 min and absorbance was measured at 535 nm. Results were expressed as micromole TBARS formed/h/g tissue.

2.6.4. Protein carbonyl (PCC) content

Protein carbonyl content was estimated according to the protocol of Hawkins *et al.* [36]. Homogenate was diluted to a protein concentration ~1 mg/ml. A sample of ~250 μl of each diluted homogenate was taken in an Eppendorf centrifuge tube. 2,4-Dinitrophenylhydrazine, 10 mM, in 2.5 M HCl; 250 μl; was added. The sample was vortexed and kept in the dark for 20 min. trichloroacetic acid (TCA; 50 % (w/v), ~ 125 μl, was added, mixed thoroughly and incubated for 15 min at −20 °C. The tubes were then centrifuged at 4 °C; 10 min; 9000 rpm. The supernatant was discarded and the pellet was washed twice with ice cold ethanol: ethyl acetate (1:1). Finally, the pellets were re-dissolved in 6 M guanidine HCl, 1 ml, and absorbance was read at 370 nm.

2.6.5. Spectrophotometric assay for caspase-9 (Dronc) and caspase-3 (Drice) activities

The assay was performed according to the manufacturer's protocol with some modification (Bio-Vision, Milpitas, CA). The assay was based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) obtained after specific action of caspase-3 and caspase-9 on tetrapeptide substrates, DEVD-pNA and LEHD-pNA, respectively. The assay mixture consisted of cell suspension, 50 μl, and chilled cell lysis buffer, incubated on ice for 10 min. After incubation, 2X reaction buffer (containing 10 mM DTT), 50 μl, with 200 μM substrate (DEVD-pNA for Drice, and IETD-pNA for Dronc) was added and incubated at 37 °C for 1.5 h. The reaction was quantified at 405 nm.

2.6.6. Superoxide dismutase activity

The method of Marklund and Marklund [37] was used. Reaction mixtures consisted of sample, 17 μl, and 0.1 M phosphate buffer, 950 μl. The reaction was initiated by adding pyrogallol (4.5 mM). Absorbance at 420 nm was recorded every 30 s for 3 min and results were expressed as units/mg protein.

2.6.7. Catalase activity

Activity was estimated using the method of Beers and Sizer [38]. Homogenate (~50 μl) was mixed with 0.1 mol/l phosphate buffer, 438 μl, and 0.5 mol/l H_2O_2, 250 μl was added and absorbance was read at 240 nm. Activity was expressed as μmol of H_2O_2 consumed/min/mg protein.

2.7. Effect on pupation and emergence of flies

Fifty first-instar larvae were introduced in the vials containing the desired concentration of $AlCl_3$. The numbers of pupae followed by the numbers of emerged flies were recorded in the control and treated groups for 20 days. Three sets of each treatment were employed in the study. From the fourth day, numbers of larvae pupate followed by number of emerging flies were recorded separately. Data was expressed as the mean of three replicates (50 larvae/replicate) [39].

2.8. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, by using GraphPad Prism software [version 5.0]. For pupation and emergence long run test was used for the analysis. Significance level was set as $p < 0.05$.

3. Results

Dose-dependent increases, significant relative to the controls at all $AlCl_3$ doses tested, were observed in the following indicators of toxicity: GST activity (Fig. 4b); caspase-3 activity (Fig. 4d); SOD activity (Fig. 4f). For the following indicators of toxicity, increases, significant relative to the controls, were observed at 40 μM $AlCl_3$ doses and higher, although not at the lowest (20 μM dose: β-galactosidase activity (Fig. 1); X-gal staining (Fig. S1); tissue damage (Fig. S2 and Fig.)2); DNA damage (midgut cells of third-instar larvae, Fig. 3a and b; Fig. 4a); PCC (Fig. 4c; caspase-9 activity; catalase activity (Fig. 4g); TBARS (Fig. 4h).

Results for the rate of pupation and emergence of flies are shown (Fig. S3a and b). Pupation began on the fifth day in both the exposed and control groups. A dose-dependent delay in the rate of pupation was observed in larvae exposed to doses > 20 μM. Emergence began from day 12 onward in both exposed and control groups. A dose-dependent and significant reduction in emergence rate was observed in larvae exposed to dose > 20 μM.

4. Discussion

We have confirmed the toxicity of $AlCl_3$ in third-instar larvae of transgenic *Drosophila melanogaster (hsp-70 lac Z) Bg*[9]. Al (Al) is one of the most widespread metals in the Earth's crust but it has no known essential role in metabolism [3]. Al exposure has been linked to the onset and progression of neurological disorders, including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS) [40–42]. $AlCl_3$ has been reported to increase oxidative stress and reduce antioxidant defense mechanisms [43–44]. Here, we found a reduction in GSH content and increases in GST, TBARS, PCC, CAT and SOD activities. Delay in pupation and emergence was observed. Oxidative stress arises when generation of reactive oxygen species (ROS) exceeds capacity to counteract their harmful effects. Such an imbalance can result in extensive cellular injury, including membrane disruption, protein alterations, DNA damage, and eventually cell death [45].

We observed a significant increase in β-galactosidase activity following exposure to $AlCl_3$, indicating cytotoxicity. As a reporter gene, β-galactosidase provides an indirect measure of *hsp70* expression. Typically, the induction of *hsp70* by environmental chemicals is associated with early cellular stress and cytotoxic responses. Several studies have employed β-galactosidase activity as a marker for *hsp70* expression under stress conditions [46]. The X-gal staining results were consistent with the findings of the ONPG assay, with the highest β-galactosidase activity observed in larvae exposed to 80 μM $AlCl_3$. The use of β-galactosidase activity as a marker of *hsp70* expression in response to cadmium toxicity has been investigated in transgenic *Caenorhabditis elegans*. The ONPG assay was selected because it provides a sensitive, quantitative,

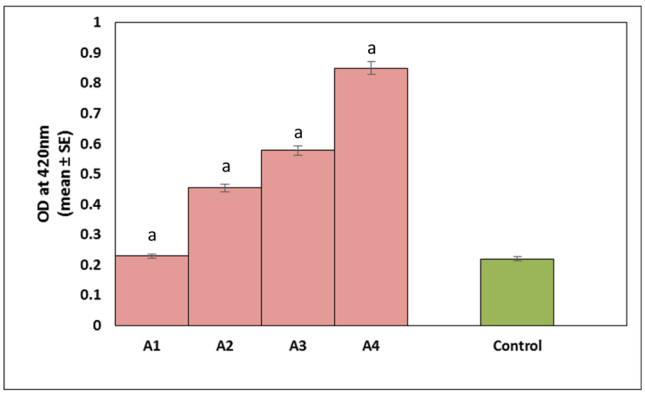

Fig. 1. β-galactosidase activity measured in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg*[9] exposed to various doses of Aluminum chloride ($AlCl_3$) for 24 hrs of duration. [a]significant at $p < 0.05$ compared to control [A-Aluminum chloride; A1–20 μM; A2–40 μM; A3–60 μM; A4–80 μM].

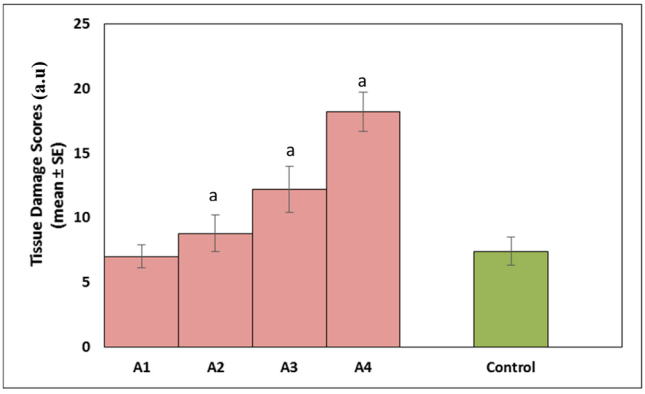

Fig. 2. Quantification of the tissue damage observed in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg9* exposed to various doses of Aluminum chloride ($AlCl_3$) for 24 hrs of duration. [a]significant at $p < 0.05$ compared to control. [A-Aluminum chloride; A1–20 μM; A2–40 μM; A3–60 μM; A4–80 μM].

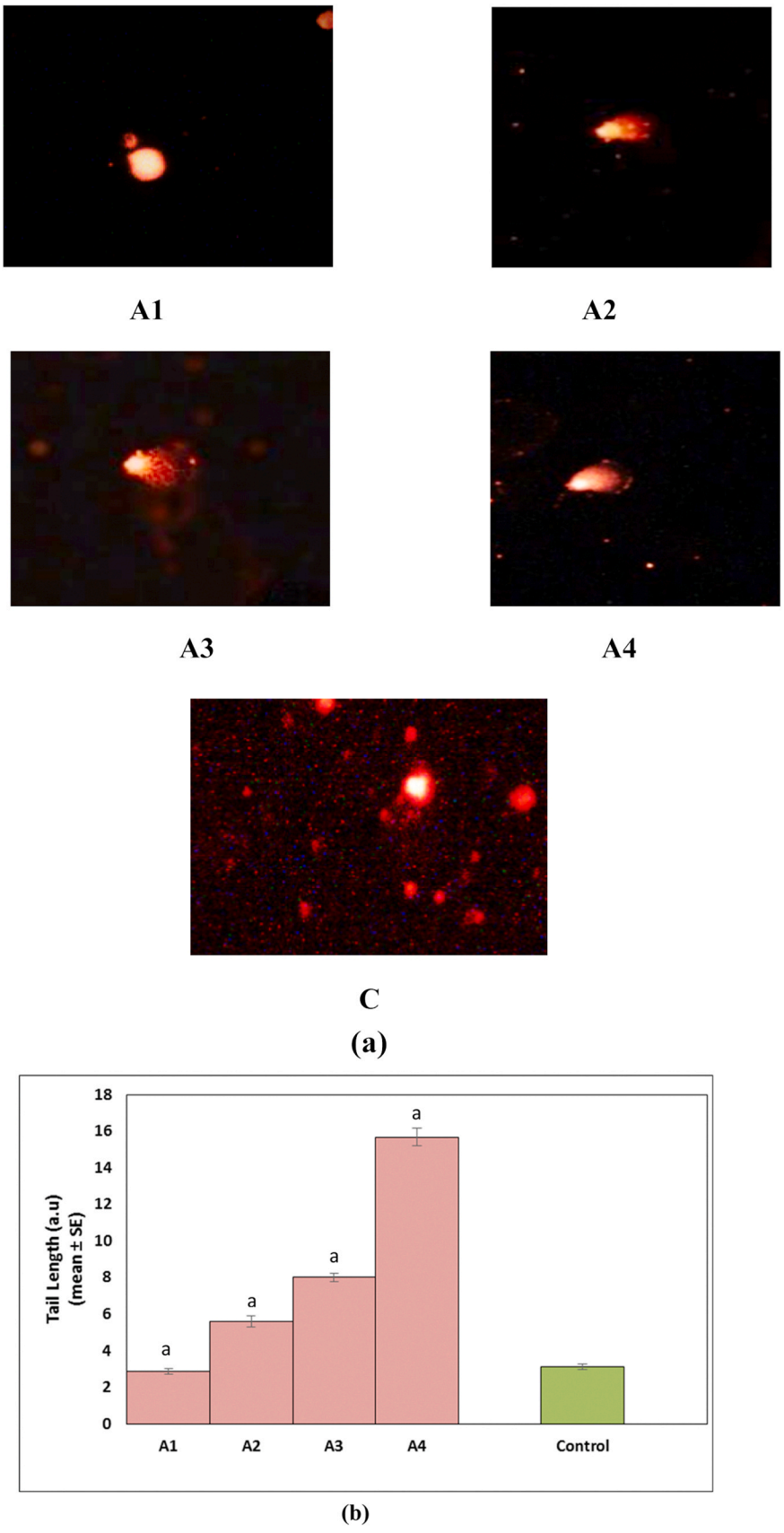

Fig. 3. (a) Comet assay performed on the midgut cells of the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg*^{9} exposed to various doses of Aluminum chloride ($AlCl_3$) for 24 hrs of duration (a-e) **(b)** quantification of the DNA damage. [a]significant at $p < 0.05$ compared to control. [A-Aluminum chloride; A1–20 μM; A2–40 μM; A3–60 μM; A4–80 μM].

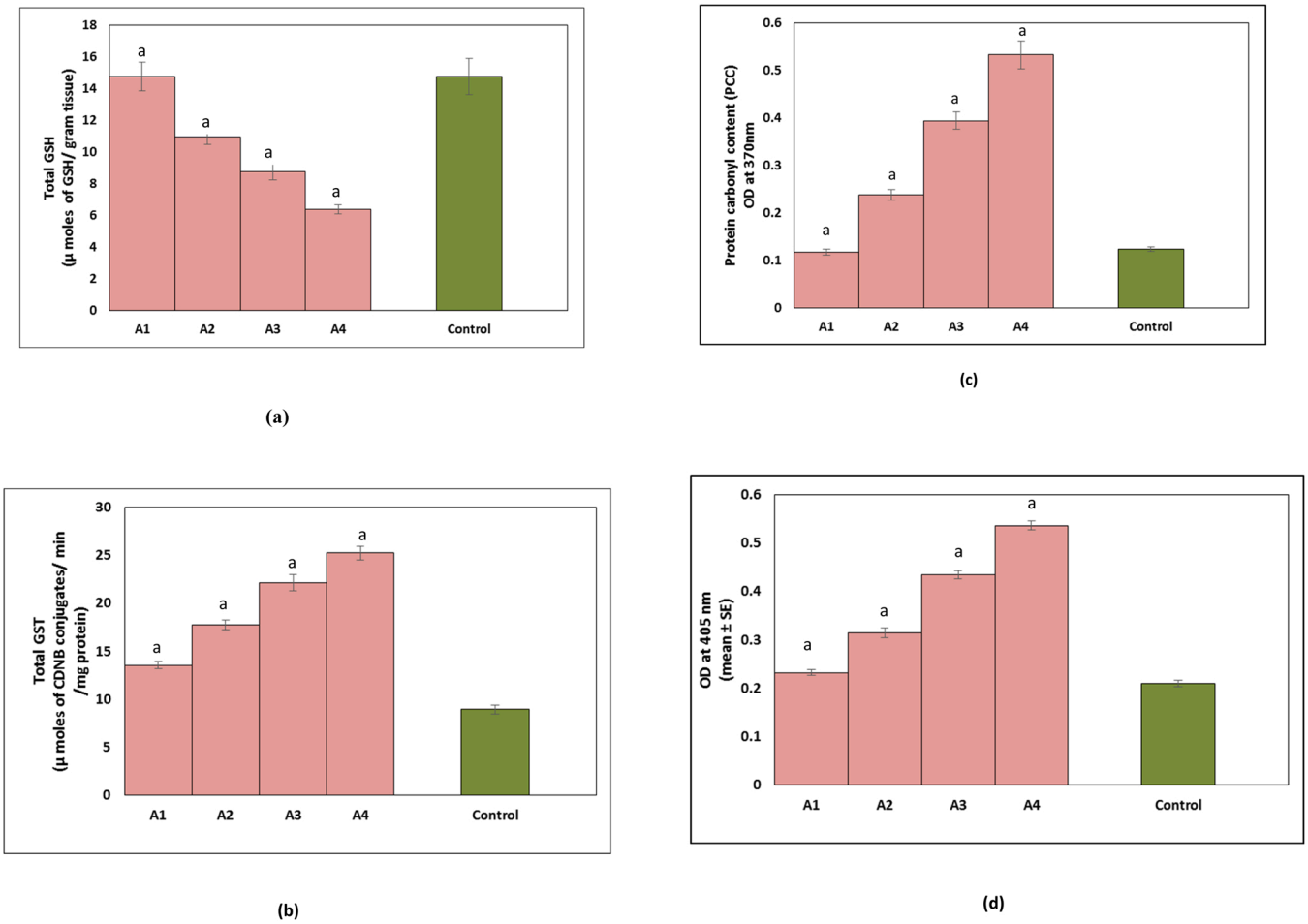

Fig. 4. Glutathione (GSH) (a), Glutathione S Transferase (GST) (b), protein carbonyl content (PCC) (c), Caspase-3 (d), Caspase-9 (e), Superoxide dismutase (SOD) (f), Catalase (CAT) (g), Lipid peroxidation (LPO) (h) activity measured in the third instar larvae of transgenic *Drosophila melanogaster (hsp70-lacZ) Bg*^{9} exposed to various doses of A-Aluminum chloride ($AlCl_3$) for 24 h of duration. [a]significant at $p < 0.05$ compared to control [A-Aluminum chloride; A1–20 µM; A2–40 µM; A3–60 µM; A4–80 µM; Data presented as mean±SE].

and reproducible measure of β-galactosidase activity, which serves as an indirect but highly reliable marker of cellular stress in the hsp70-lacZ transgenic *Drosophila* line. In this system, the lacZ reporter gene is transcriptionally fused to the hsp70 promoter; therefore, any stress-induced activation of hsp70 in response to toxic exposure results in proportional expression of β-galactosidase. β-Galactosidase levels thus accurately reflect the extent of hsp70 induction, making it a dependable biomarker of toxicity [47]. Because hsp70 is a highly conserved and rapidly inducible heat-shock protein that responds to protein denaturation, oxidative imbalance, and general cellular injury, monitoring β-galactosidase activity through the ONPG assay provides a robust readout of toxicant-mediated stress [48]. The typan blue exclusion assay was performed to detect tissue damage in third instar *Drosophila* larvae, showing a significant dose-dependent increase following $AlCl_3$ exposure. The association between *hsp70* expression and tissue damage has been well established [29]. We also observed a significant increase in tissue damage compared following exposure of *Drosophila* larvae to $AlCl_3$.

The comet assay (single-cell gel electrophoresis assay) is a sensitive method for visualizing DNA breaks in single-cell suspension [49]. It detects DNA strand breaks, alkali-labile sites, and other forms of DNA damage [50–51]. The cellular target for performing comet assay in the larvae was the midgut, as most toxic substances enter the body via food into the gut.

Glutathione (GSH) is a tripeptide involved in diverse biological processes, including enzymatic reactions, molecular transport, biosynthesis of proteins and nucleic acids, microtubule assembly, signal transduction, gene regulation, and defense against oxidative stress [52]. As one of the major endogenous antioxidants, GSH defends against free radicals and reactive oxygen species [53]. Under oxidative stress, intracellular GSH levels decline [54]. GST activity is dependent on a constant supply of GSH, as its main function is to detoxify xenobiotics by catalyzing the nucleophilic attack of GSH on electrophilic groups. During oxidative stress, GST activity has been reported to increase as part of the detoxication response. Akano et al. [55] showed that GSH level decreases following exposure to $AlCl_3$ whereas Ogunsuyi et al. [56] showed a significant increase in GST activity on exposure to $AlCl_3$. Inneh and Eiya [57] reported that exposing *Drosophila* flies to 40 mM of $AlCl_3$ for about 7 days led to a decline in GST activity. Lipid peroxidation serves as an indicator of oxidative stress, arising from the generation of reactive oxygen species (ROS) that cause damage to cellular membranes, proteins, and DNA. Malondialdehyde (MDA) is the principal reactive aldehyde generated during the peroxidation of biological membranes [58]. It can interact with DNA, forming adducts, as well as with proteins, RNA, and other biomolecules [59]. In the present study, MDA levels were quantified TBARS assay. In a previous study [60], an increase in LPO was found when rats were administered to 34 mg/Kg/bw $AlCl_3$ for about 70 d. Similar to the previous study, we found a dose-dependent increase in LPO in the larvae exposed to $AlCl_3$, confirming the production of increased ROS. Protein oxidation is considered one of the earliest

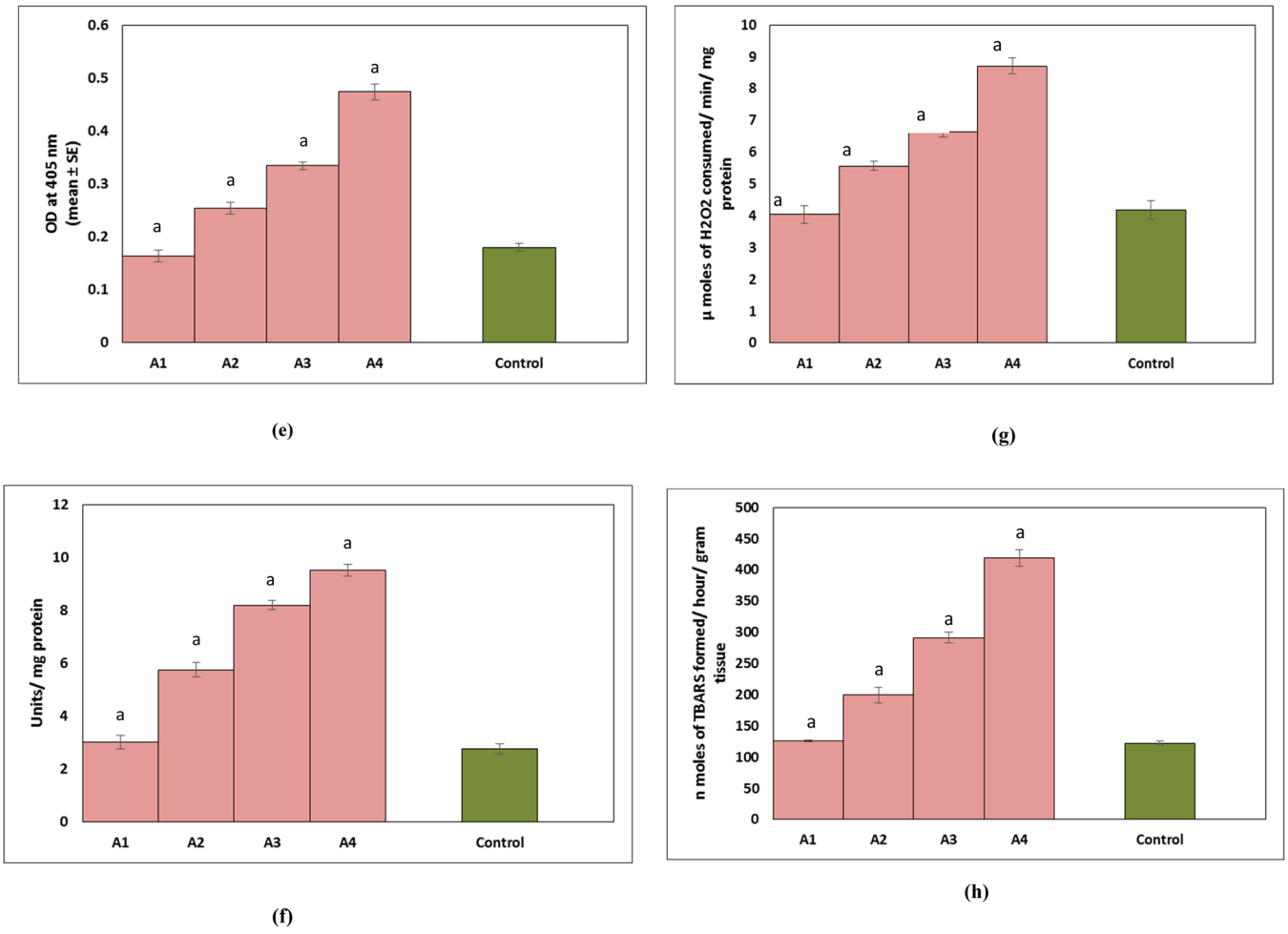

Fig. 4. (*continued*).

manifestations of oxidative damage and can be assessed through protein carbonyl content (PCC) [61]. Oyetayo et al. [62] observed an increase in PCC on following exposure of Drosophila to 100–200 mg/kg of $AlCl_3$ for 7 d. In the present study, similar results were found when larvae were exposed to $AlCl_3$. Caspases are well-conserved cysteine proteases that play a central role in regulating apoptosis. In *Drosophila*, Drice and Dronc serve as the functional counterparts of Caspase-3 and Caspase-9, respectively. To assess further the genotoxicity of $AlCl_3$, we performed the assay on midgut cells, finding an increase in the activities of caspase 3 and 9 and further confirming the toxicity of $AlCl_3$. Superoxide dismutase (SOD) is a crucial antioxidant enzyme that protects cells from oxidative stress by catalyzing the conversion of the superoxide radical into molecular oxygen and hydrogen peroxide [63]. SOD helps maintain cellular redox balance and prevents oxidative damage to proteins, lipids, and DNA. This enzyme plays a vital role in cellular defense mechanisms, aging, inflammation, and the pathophysiology of diseases associated with oxidative stress. In our study, we observed an increase in the level of SOD following exposure of larvae to $AlCl_3$, indicating that Al may interfere with antioxidant activity by suppressing antioxidant enzymes, and aligning with findings from other studies [60,64]. Catalase (CAT) is a crucial intracellular antioxidant enzyme located in the peroxisomes of aerobic cells. It plays an essential role in protecting cells from oxidative stress. We found that activity of CAT increased following exposure of larvae to $AlCl_3$ and implying that Al may impair antioxidant defense by reducing CAT activity, consistent with previous studies [65–66].

Exposure to $AlCl_3$ resulted in a reduction in emergence and developmental rate. $AlCl_3$ can cause a range of complex functional and developmental impairments as well as interfering with signaling processes [67–68]. The transformation rate from larvae to pupae and from pupae to adults decreased in a dose-dependent manner. In insects, the midgut is regarded as the most metabolically active region. In *D. melanogaster* larvae, imidacloprid is metabolized in the midgut, and its metabolites are rapidly excreted. In vivo xenobiotic metabolism in insects is likely to be highly complex [69]. For many compounds, the resulting metabolites have been reported to exhibit greater toxicity than the parent substances. At higher doses, the larvae failed to pupate, resulting in a significant reduction in the mean number of pupae formed. Likewise, the decreased rate of pupae developing into adults may be attributed to Al, at elevated concentrations, disrupting the activity of essential enzymes required for hormone production during metamorphosis.

5. Conclusions

Exposure of third-instar larvae to $AlCl_3$ caused toxic effects spanning development, physiology, and cellular redox balance. Developmentally, $AlCl_3$ delays pupation and reduces adult emergence, indicating interference with growth and metamorphosis. At the biochemical level, exposed flies consistently exhibit oxidative stress, evidenced by elevated lipid peroxidation, protein oxidation, and ROS generation, along with depletion of reduced glutathione, thiols, and antioxidant enzymes such as SOD, CAT, and GST. These changes coincide with histological and functional indicators of tissue and neuronal damage. In addition, $AlCl_3$ exposure increases β-galactosidase activity and ONPG hydrolysis, reflecting lysosomal destabilization and a senescence-like response.

CRediT authorship contribution statement

Smita Jyoti: Software, Formal analysis. **Javeria Fatima:** Investigation. **Yasir Hasan Siddique:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Tanishka Gupta:** Investigation. **Iqra Subhan:** Writing – original draft, Visualization, Investigation, Data curation. **Kajal Gaur:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mrgentox.2025.503905.

Data availability

Data will be made available on request.

References

[1] A.A. Yaroshevsky, Abundances of chemical elements in the Earth's crust, Geochem. Int. 44 (2006) 48–55.
[2] D. Brough, H. Jouhara, The aluminium industry: a review on state-of-the-art technologies, environmental impacts and possibilities for waste heat recovery, Int. J. Thermofluids 1 (2020) 100007.
[3] C. Exley, Human exposure to aluminium, Environ. Sci. Process. Impacts 15 (2013) 1807–1816.
[4] M.J. Mold, C. Exley, Aluminium co-localises with Biondi ring tangles in Parkinson's disease and epilepsy, Sci. Rep. 12 (2022) 1465.
[5] J.L. GregerDietary and other sources of aluminium intake. In: Proceedings of the Ciba Foundation Symposium 169-Aluminium in Biology and Medicine: Aluminium in Biology and Medicine: Ciba Foundation Symposium 169: (26-49)2007.
[6] J. Suomi, P. Tuominen, Cumulative risk assessment of the dietary heavy metal and aluminum exposure of Finnish adults, Environ. Monit. Assess. 195 (2023) 809.
[7] T. Stahl, H. Taschan, H. Brunn, Aluminium content of selected foods and food products, Environ. Sci. Eur. 23 (2011) 37.
[8] H. Shirkhanloo, M.K. Abbasabadi, F. Hosseini, A.F. Zarandi, Nanographene oxide modified phenyl methanethiol nanomagnetic composite for rapid separation of aluminum in wastewaters, foods, and vegetable samples by microwave dispersive magnetic micro solid-phase extraction, Food Chem. 347 (2021) 129042.
[9] N. Hue, Soil acidity: Development, impacts, and management. Structure and Functions of Pedosphere, Springer Nature Singapore, Singapore, 2022, pp. 103–131.
[10] M.W. Jones, M.D. de Jonge, S.A. James, R. Burke, Elemental mapping of the entire intact Drosophila gastrointestinal tract, J. Biol. Inorg. Chem. 20 (2015) 979–987.
[11] J.V. Llorens, M.D. Moltó, *Drosophila melanogaster* models of metal-related human diseases and metal toxicity, Met. Metab. Anim. 18 (1456) (2018) 316.
[12] Ejim, A.I., 2006. Bioavailability and toxicity of aluminium to the freshwater pond snail, Lymnaea stagnalis at neutral pH. University of Manchester (United Kingdom).
[13] K.N. White, A.I. Ejim, R.C. Walton, A.P. Brown, R. Jugdaohsingh, J.J. Powell, C. R. McCrohan, Avoidance of aluminum toxicity in freshwater snails involves intracellular silicon– aluminum biointeraction, Environ. Sci. Technol. 42 (2008) 2189–2194.
[14] K. Woodburn, R. Walton, C. McCrohan, K. White, Accumulation and toxicity of aluminium-contaminated food in the freshwater crayfish, *Pacifastacus leniusculus*, Aquat. Toxicol. 105 (2011) 535–542.
[15] K.E. Page, K.N. White, C.R. McCrohan, D.W. Killilea, G.J. Lithgow, Aluminium exposure disrupts elemental homeostasis in *Caenorhabditis elegans*, Metallomics 4 (2012) 512–522.
[16] L. Zhao, J.P. Qiu, Aluminum bioaccumulation in the earthworm and acute toxicity to the earthworm (June). Proceedings of the 2010 4th International Conference on Bioinformatics and Biomedical Engineering (1-4), IEEE, 2010.
[17] L. Macomber, R.P. Hausinger, Mechanisms of nickel toxicity in microorganisms, Metallomics 3 (2011) 1153–1162.
[18] J.M. Gutteridge, B. Halliwell, Mini-review: oxidative stress, redox stress or redox success? Biochem. Biophys. Res. Commun. 502 (2018) 183–186.
[19] J.A. Saliu, A.M. Olajuyin, S.F. Olowolayemo, Ameliorative role of diets fortified with *Artocarpus altilis* in a *Drosophila melanogaster* model of aluminum chloride-induced neurotoxicity, Clin. Phytosci. 10 (2024) 2.
[20] Laxmi Sen Thakuri, Jung Eun Kim, Jin Yeong Choi, Dong Young Rhyu, Protective effect of *Capsosiphon fulvescens* on oxidative stress-stimulated neurodegenerative dysfunction of PC12 cells and zebrafish larva models, Fish. Aquat. Sci. 26 (2023) 24–34.
[21] Y. Cho, C.M. Park, Y.J. Heo, H.B. Park, M.S. Kim, *Drosophila melanogaster* as potential alternative animal model for evaluating acute inhalation toxicity, J. Toxicol. Sci. 49 (2024) 49–53.
[22] S. casas-tintó, drosophila as a model for human disease: insights into rare and ultra-rare diseases, Insects 15 (2024) 870.
[23] E. Tsintzas, T. Niccoli, Using Drosophila amyloid toxicity models to study Alzheimer's disease, Ann. Hum. Genet. 88 (2024) 349–363.
[24] Z. Huang, X. Dai, B. Jiang, Y. Kong, Model organisms in neuroinflammation and neuropathy: drosophila melanogaster, Front. Aging Neurosci. 16 (2024) 1502502.
[25] R. Hosamani, Muralidhara, Acute exposure of Drosophila melanogaster to paraquat causes oxidative stress and mitochondrial dysfunction, Arch. Insect Biochem. Physiol. 83 (2013) 25–40.
[26] L.T. Reiter, L. Potocki, S. Chien, M. Gribskov, E. Bier, A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*, Genome Res. 11 (2001) 1114–1125.
[27] J.T. Lis, J.A. Simon, C.A. Sutton, New heat shock puffs and β-galactosidase activity resulting from transformation of *Drosophila* with an hsp70-lacZ hybrid gene, Cell 35 (1983) 403–410.
[28] D.K. Chowdhuri, D.K. Saxena, P.N. Viswanathan, Effect of hexachlorocyclohexane (HCH), its isomers, and metabolites on Hsp70 expression in Transgenic *Drosophila melanogaster*, Pestic. Biochem. Physiol. 63 (1999) 15–25.
[29] A. Nazir, I. Mukhopadhyay, D.K. Saxena, M.S. Siddiqui, D.K. Chowdhuri, Evaluation of toxic potential of captan: Induction of hsp70 and tissue damage in transgenic Drosophila melanogaster (hsp70-lacZ) Bg^9, J. Biochem. Mol. Toxicol. 17 (2003) 98–107.
[30] K. Gaur, H. Varshney, I. Subhan, J. Fatima, S. Jyoti, Y.H. Siddique, Evaluation of Bis (2-ethylhexyl) phthalate toxicity on the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9, Food Chem. Toxicol. 184 (2024) 114425.
[31] R.A. Krebs, M.E. Feder, Tissue-specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae, J. Exp. Biol. 200 (1997) 2007–2015.
[32] I. Mukhopadhyay, D.K. Chowdhuri, M. Bajpayee, A. Dhawan, Evaluation of in vivo genotoxicity of cypermethrin in *Drosophila melanogaster* using the alkaline Comet assay, Mutagenesis 19 (2004) 85–90.
[33] D.J. Jollow, J.R. Mitchell, N.A. Zampaglione, J.R. Gillette, Bromobenzene-induced liver necrosis. protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite, Pharmacology 11 (1974) 151–169.
[34] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione S-transferases: the first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[35] H. Ohkawa, N. Ohishi, K. Yagi, Reaction of linoleic acid hydroperoxide with thiobarbituric acid, J. Lipid Res. 19 (1978) 1053–1057.
[36] C.L. Hawkins, P.E. Morgan, M.J. Davies, Quantification of protein modification by oxidants, Free Radic. Biol. Med. 46 (2009) 965–988.
[37] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (1974) 469–474.
[38] R.F. Beers, I.W. Sizer, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem. 195 (1952) 133–140.
[39] S. Podder, S. Roy, Study of the changes in life cycle parameters of *Drosophila melanogaster* exposed to fluorinated insecticide, cryolite, Toxicol. Ind. Health (2015) *31*1341–311347.
[40] W.R. Harris, Z. Wang, Y.Z. Hamada, Competition between transferrin and the serum ligands citrate and phosphate for the binding of aluminium, Inorg. Chem. 42 (2003) 3262–3273.
[41] S. Oshiro, M. Kawahara, Y. Kuroda, C. Zhang, Y. Cai, S. Kitajima, M. Shirao, Glial cells contribute more to iron and aluminum accumulation but are more resistant to oxidative stress than neuronal cells, Biochim. Biophys. Acta Mol. Basis Dis. 1502 (2000) 405–414.
[42] S. Davenward, P. Bentham, J. Wright, P. Crome, D. Job, A. Polwart, C. Exley, Silicon-rich mineral water as a non-invasive test of the "aluminum hypothesis" in Alzheimer's disease, J. Alzheimer's. Dis. 33 (2013) 423–430.
[43] M.I. Yousef, K.I. Kamel, M.I. El-Guendi, F.M. El-Demerdash, An in vitro study on reproductive toxicity of aluminium chloride on rabbit sperm: the protective role of some antioxidants, Toxicology 239 (2007) 213–223.
[44] W.A. Moselhy, N.A. Helmy, B.R. Abdel-Halim, T.M. Nabil, M.I. Abdel-Hamid, Role of ginger against the reproductive toxicity of aluminium chloride in albino male rats, Reprod. Domest. Anim. 47 (2012) 335–343.
[45] M. Aboubakr, M. Elbadawy, S.S. Ibrahim, E. Khalil, M. Darweish, A. Farag, A. Elfadadny, M. Alkafafy, A. Soliman, A. Elsayed, Allicin and lycopene possesses a protective effect against methotrexate-induced testicular toxicity in rats, Pak. Vet. J. 43 (2023) 559–566.
[46] A. Elsayed, A. Elkomy, M. Alkafafy, R. Elkammar, S.E. Fadl, E.Y. Abdelhiee, A. Abdeen, G. Youssef, H. Shaheen, A. Soliman, Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats, Pak. Vet. J. 42 (2022) 107–111.

[47] Y.H. Siddique, M.S. Ansari, Rahul, S. Jyoti, Effect of alloxan on the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9, Toxin Rev. 39 (2020) 41–51.
[48] L.K. Cioci, L. Qiu, J.H. Freedman, Transgenic strains of the nematode *Caenorhabditis elegans* as biomonitors of metal contamination, Environ. Toxicol. Chem. 19 (8) (2000) 2122–2129.
[49] A. Dhawan, M.M. Bajpayee, A.K. Pandey, D. Parmar, Protocol for the single cell gel electrophoresis/comet assay for rapid genotoxicity assessment, Sigma 1077 (1) (2003) 1–10.
[50] W. Liao, M.A. McNutt, W.G. Zhu, The comet assay: a sensitive method for detecting DNA damage in individual cells, Methods 48 (2009) 46–53.
[51] P. Rajak, M. Dutta, S. Khatun, M. Mandi, S. Roy, Exploring hazards of acute exposure of Acephate in *Drosophila melanogaster* and search for l-ascorbic acid mediated defense in it, J. Hazard. Mater. 321 (2017) 690–702.
[52] H. Jefferies, J. Coster, A. Khalil, J. Bot, R.D. McCauley, J.C. Hall, Glutathione, ANZ J. Surg. 73 (2003) 517–522.
[53] M. Danish, A. Fatima, S. Khanam, S. Jyoti, F. Ali, F. Naz, Y.H. Siddique, Evaluation of the toxic potential of calcium carbide in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lacZ) Bg9, Chemosphere 139 (2015) 469–478.
[54] Y.H. Siddique, S. Jyoti, Alteration in biochemical parameters in the brain of transgenic *Drosophila melanogaster* model of Parkinson's disease exposed to apigenin, Integr. Med. Res. 6 (3) (2017) 245–253.
[55] J.J.M. Akano, Z.A. Molik, A.O. Abolaji, O.O. Ogbole, Mitigating aluminum chloride-induced toxicity in *Drosophila melanogaster* with peptide fractions from Euphorbia species, Drug Chem. Toxicol. 48 (3) (2025) 687–696.
[56] O.B. Ogunsuyi, O.C. Olagoke, B.A. Afolabi, G. Oboh, O.M. Ijomone, N.V. Barbosa, J.B. da Rocha, Dietary inclusions of Solanum vegetables mitigate aluminum-induced redox and inflammation-related neurotoxicity in *Drosophila melanogaster* model, Nutr. Neurosci. 25 (10) (2022) 2077–2091.
[57] C.A. Inneh, B.O. Eiya, Anticholinesterase activity and antioxidant effect of vitamin E in aluminium chloride induced toxicity in *Drosophila melanogaster*, J. Afr. Assoc. Physiol. Sci. 11 (1) (2023) 17–28.
[58] M.E. Iyiegbu, A.B. Enogieru, Antioxidant and anticholinesterase activity of rutin in aluminum chloride-exposed *Drosophila melanogaster*, Comp. Clin. Pathol. 33 (3) (2024) 445–452.
[59] Y.H. Siddique, S. Jyoti, F. Naz, Protective effect of luteolin on the transgenic Drosophila model of Parkinson's disease, Braz. J. Pharm. Sci. 54 (03) (2018) e17760.
[60] A.S.A. Newairy, A.F. Salama, H.M. Hussien, M.I. Yousef, Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats, Food Chem. Toxicol. 47 (6) (2009) 1093–1098.
[61] I. Dalle-Donne, D. Giustarini, R. Colombo, R. Rossi, A. Milzani, Protein carbonylation in human diseases, Trends Mol. Med. 9 (2003) 169–176.
[62] B.O. Oyetayo, A.O. Abolaji, K.D. Fasae, A. Aderibigbe, Ameliorative role of diets fortified with curcumin in a *Drosophila melanogaster* model of aluminum chloride-induced neurotoxicity, J. Funct. Foods 71 (2020) 104035.
[63] I. Subhan, S. Khan, H. Varshney, K. Gaur, Y.H. Siddique, Effect of geraniol against the toxicity induced by paracetamol in the third instar larvae of transgenic *Drosophila melanogaster* (hsp70-lac Z) Bg9, Curr. Drug Ther. 19 (2024) 851–862.
[64] J.O. Efosa, K. Omage, M.A. Azeke, *Hibiscus sabdariffa* calyx protect against oxidative stress and aluminium chloride-induced neurotoxicity in the brain of experimental rats, Toxicol. Rep. 10 (2023) 469–480.
[65] P. Bhalla, D. Dhawan, Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain, Cell Mol. Neurobiol. 29 (4) (2009) 513–521.
[66] L. Liaquat, S. Sadir, Z. Batool, S. Tabassum, S. Shahzad, A. Afzal, S. Haider, Acute aluminum chloride toxicity revisited: study on DNA damage and histopathological, biochemical and neurochemical alterations in rat brain, Life Sci. 217 (2019) 202–211.
[67] S.Z. Raduan, Q.U. Ahmed, A.R. Kasmuri, M.R.A. Rusmili, W.A.W. Sulaiman, M. F. Shaikh, M.H. Mahmood, S.N.H. Azmi, M.Z. Ahmed, S. Kazmi, Neurotoxicity of aluminium chloride and okadaic acid in zebrafish: insights into Alzheimer's disease models through anxiety and locomotion testing, and acute toxicity assessment with *Litsea garciae* bark's methanolic extract, J. King Saud. Univ. Sci. 35 (7) (2023) 102807.
[68] A. Sudwarts, Zebra Fish as a Model for Translational Neurobiology: Implications for Drug Discovery and Development, Queen Mary University of London, 2017.
[69] Anushree, M.Z. Ali, A.L. Bilgrami, J. Ahsan, Acute exposure to arsenic affects pupal development and neurological functions in *Drosophila melanogaster*, Toxics 11 (2023) 327.