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Low molecular weight chitosan attenuates acrylamide-induced toxicity in *Drosophila melanogaster*

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ABSTRACT

Acrylamide (ACR), a toxic by-product of high-temperature food processing, poses significant health risks due to its oxidative, neurotoxic, and genotoxic properties. Regulatory measures focus on limiting ACR in commercial food products, yet daily cooking practices often result in unnoticed exposure, threatening vulnerable populations such as children. This study evaluates the protective role of low and medium molecular-weight (MW) chitosan against ACR-induced toxicity using *Drosophila melanogaster*. Chitosan, a natural polysaccharide with antioxidant and prebiotic properties, was supplemented alongside ACR exposure in larvae and adult flies. Developmental metrics such as pupation rates, fecundity, and adult emergence were assessed, alongside oxidative stress markers and neurobehavioral outcomes. ACR exposure impaired development, increased oxidative stress, and reduced locomotor activity. Supplementation with low and medium MW chitosan alleviated these effects, with low MW chitosan demonstrating greater efficacy. These findings reveal the potential of low MW chitosan as a dietary intervention to counteract the toxic effects of contaminants like ACR. By reducing oxidative stress, preserving mitochondrial function, and supporting developmental processes, chitosan offers a promising avenue for mitigating the overall toxicity of heat-processed toxins. These findings further highlight chitosan's molecular weight-dependent protective potential against ACR toxicity, offering insights into its application as a dietary mitigator of heat-processed toxins.

1. Introduction

Sedentary lifestyles, imbalanced diets, and chronic stress have all contributed to the growth of noncommunicable illnesses such as obesity, diabetes, and metabolic disorders. Environmental variables, such as dietary heat-process contaminants like acrylamide (ACR), have also exacerbated these health hazards [1]. Despite regulatory efforts to reduce ACR in commercial food items, its production during household preparation continues undiminished, providing considerable health risks, particularly to vulnerable populations such as children. ACR toxicity has been associated with oxidative stress, neurotoxicity, and genotoxicity, and prolonged exposure increases the risk of neurological and reproductive system deterioration [2–4]. Current mitigation measures, such as enzymatic treatments and advanced thermal procedures, have limits in scalability, cost, and practicality, emphasising the need for new approaches.

ACR-induced toxicity is also closely linked to gut microbiota dysbiosis, leading to systemic oxidative stress and inflammation, though the underlying mechanisms remain poorly understood. Animal studies highlight ACR's effects on the gut-brain axis, including cognitive impairments, intestinal barrier dysfunction, microbial imbalances, and altered bile acid metabolism [5–7]. Additionally, ACR disrupts intestinal morphology, damages the enteric nervous system, increases apoptotic markers, aggravates mucosal inflammation, and compromises tight junction integrity [8–11]. Despite extensive research on ACR's neurotoxic and carcinogenic effects, developmental toxicity remains underexplored [12]. This is particularly critical as early life stages may be more susceptible to toxin-induced disruptions in gut-associated metabolic pathways, which can influence long-term growth, development, and immune competence [13–15]. Addressing these gaps by focusing on gut microbiota and developmental toxicity offers a promising avenue for understanding and mitigating ACR's multifaceted health risks.

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The traditional methods of mitigating ACR toxicity often overlook the role of gut-mediated pathways in systemic toxicity. Biopolymers like chitosan, which act as antioxidants and prebiotics, offer a promising alternative. These prebiotics can influence gut microbiota composition and activity, targeting gut-mediated pathways implicated in ACR toxicity. This can reduce systemic oxidative stress, restore gut homeostasis, and enhance host resilience to toxicants [15,16]. Integrating gut-focused mitigation strategies using prebiotic biopolymers is a critical and underexplored aspect of food toxicology, potentially leading to innovative therapeutic approaches. This study addresses these critical research gaps in the domain of ACR toxicity. While focusing on multiple endpoints, including behavioural and biochemical aspects, it also investigates the efficacy of the proposed chitosan-based therapeutic approach in mitigating the developmental and reproductive impacts of ACR within the *Drosophila* model.

Chitosan, a biopolymer derived from chitin in crustaceans, holds promise as a natural mitigator of ACR toxicity due to its unique properties, including antioxidant, anti-inflammatory, and prebiotic effects. Regulatory agencies like the FDA and EU Commission recognize chitosan as safe, making it a suitable candidate for dietary and pharmaceutical applications [17–19]. Its bioactivities are strongly influenced by molecular weight (MW) and degree of deacetylation (DA), which affect solubility, bioavailability, and biological interactions [20,21]. Low MW chitosan exhibits superior solubility and antioxidant potential, making it more effective in neutralising oxidative stress and interacting with biological systems. On the other hand, Medium MW chitosan offers enhanced structural stability, contributing to its functional versatility [22–27]. Chitosan is a naturally derived, odourless, and flavourless biopolymer that remains stable under various food processing conditions, making it an ideal choice for long-term use without affecting product taste or texture. It is cost-effective, easy to apply, and can be seamlessly incorporated into food products without complex procedures, unlike phytochemicals and enzyme-based ACR modulation strategies.

Chitosan has been found to influence the formation of ACR by modulating Maillard reaction pathways in asparagine–fructose model systems [28]. It has been used in synbiotic formulations to alleviate acrylamide-induced toxicity, and in edible coatings to reduce toxic biogenic amines in food products [16,29]. Chitosan nanoparticles have shown protective effects against toxins like Bisphenol-A, reducing oxidative stress and restoring biochemical parameters in animal models [30]. This study focuses on multi-focal endpoints including developmental and reproductive aspects of ACR toxicity, specifically within the *Drosophila melanogaster* model. This is a significant gap in the literature, as most studies focus on immediate toxicity effects, neglecting the potential impacts of ACR on growth, reproduction, and overall development. Additionally, a detailed comparison of the therapeutic potential of different MW variants is lacking in the current literature.

In vivo models are essential for assessing the biological effects of ACR and evaluating protective agents. *Drosophila melanogaster* offers a valuable platform due to its genetic similarity to higher organisms, short lifespan, and cost-effectiveness[31]. This study investigates the differential effects of low and medium MW chitosan on ACR-induced toxicity in the *Drosophila* model, addressing the knowledge gap regarding the role of molecular weight in determining chitosan's bioactivity. By elucidating the molecular weight-dependent protective effects of chitosan, this study aims to establish a foundation for developing effective, scalable, and practical interventions to mitigate ACR toxicity and its adverse health impacts.

2. Materials and methods

2.1. Chemicals

Low molecular weight chitosan (34.5 m Pas, 90.1 % degree of deacetylation, ~ 50 kDa), medium molecular weight chitosan (157 m Pas, 90.11 % degree of deacetylation, ~190 kDa), Acrylamide (3x Crystalline), glacial acetic acid, Acetylcholine iodide, yeast extract, propionic acid, orthophosphoric acid, nitro blue tetrazolium salt (NBT), Abcam TMRE-mitochondrial membrane potential assay kit, phenazonium Methosulphate (PMS), nicotinamide adenine dinucleotide (reduced) disodium salt (NADH), tetrasodium pyrophosphate (TSPP), ethanol, and 5, 5-dithiobis 2-nitrobenzoic acid (DTNB) were procured from Sisco Research Laboratories, India. Agar-Agar Type −1, dextrose, sodium phosphate monobasic, Schneider's insect medium sodium, phosphate dibasic, phosphate buffer saline (PBS) and methylparaben were purchased from Himedia Pvt. Ltd., India. All chemicals used in this study were of analytical grade.

2.2. Drosophila maintenance and treatment exposure

The *Drosophila* Oregon K wild-type strain was kept at 25 ± 2 °C and 60 % humidity under a 12-h light-dark cycle. The flies were bred on a standard cornmeal agar medium containing cornflour, agar-agar type 1, D-glucose, sugar, and yeast extract. To avoid microbial contamination, the medium was autoclaved and treated with antifungal agents, including propionic acid, Tego (methyl para-hydroxybenzoate dissolved in ethanol), and orthophosphoric acid at 55 °C. Experimental concentrations of ACR and low and medium molecular weight chitosan (LC or MC, respectively) were administered to the flies orally by stoichiometric addition of respective compounds to the media at 50–55 °C. The effects of chitosan variants against ACR were studied by combining experimentally derived concentrations of the respective compounds with treatment groups labelled as ACR+LC or ACR+MC. Baseline groups such as control, 2.5 LC, 5 MC, and solvent control (SC) – glacial acetic acid alongside toxin control (ACR) were established to emphasise the impacts of the treatment groups. The doses of chitosan and acrylamide were selected based on previously published studies and pilot experiments conducted in our laboratory. Chitosan doses (2.5–10 mg/ml) were optimized to ensure they were within a range that showed biological effects without causing overt toxicity, as detailed by Kumar et al., 2019, providing a basis for dose optimization [32]. Based on the short survival period and the toxic impacts of ACR on flies reported by Senthilkumar et al. [3], the ACR dose was set to 2 mM with a treatment exposure duration of 5 days. All the experimental analyses were performed in triplicates for statistical validity.

2.3. Survival and behavioural analysis

Survival analysis reflects the lifespan of the fruit fly model reared on the treated media, serving as a direct indicator of how the compound of interest affects the overall well-being of the flies. Thirty newly eclosed male flies were added to vials containing fresh treated media and maintained at standard rearing conditions. These flies were monitored every 24 h with periodic refreshment of the treated media every four days, and then the number of dead flies was tallied and recorded for further statistical analysis. The behavioural analysis in the fruit fly model complexly relates locomotor ability and neural signalling based on environmental-specific cues.

The larval locomotor ability was determined by analysing its crawling distance per minute. Newborn male and female flies (parent flies) (in the ratio 2:1) were exposed to the respective treatment media, and the resulting third instar larvae were made to crawl on a 2 % agar plate placed over a graph sheet. The data were then recorded for further statistical analysis. Likewise, adult locomotor functions were calculated based on their negative geotaxis ability, which was then denoted as the percentage of active flies. The treated male flies were transferred to the climbing assay vials with a 3 cm marking. The vials were then tapped for five seconds and left undisturbed, later, the number of flies above the 3 cm mark was recorded after 15 s for further analysis [16].

2.4. Biochemical analysis

Exposure to xenobiotics induces disruption in the redox mechanisms and can easily be investigated by estimating the fluctuations in redox stress and neurotoxic markers like reactive oxygen species levels (ROS), superoxide dismutase (SOD) and acetylcholine esterase (AChE) enzyme activities. The biochemical estimations were performed using thirty treated male flies following previously reported protocols [3,33]. Briefly, thirty male *Drosophila melanogaster* were exposed to their respective treatment groups for five days. To measure reactive oxygen species (ROS), the flies were homogenized, and the homogenate was centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was treated with 10 mM 2,7-Dichlorofluorescein diacetate (DCHF-DA) and incubated at room temperature for 60 min. Fluorescence was then measured using excitation at 488 nm and emission at 525 nm. Acetylcholinesterase (AChE) activity was assessed by homogenizing thirty male flies, followed by treatment with 10 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and acetylcholine iodide. The absorbance was recorded at 412 nm over a 10-min period, with measurements taken every minute. For superoxide dismutase (SOD) activity, the flies were exposed to their respective treatments for five days, and their homogenates were centrifuged at 6000 rpm for 10 min at 4 °C. SOD activity was determined using a reaction mixture containing NADH, sodium pyrophosphate buffer, nitroblue tetrazolium, and phenazine methosulfate, as described by Ahmed et al. After 1 min, the reaction was halted with glacial acetic acid, and the absorbance was measured at 560 nm.

2.5. Estimation of developmental factors

Within the fruit fly model, the developmental factors denote a variety of parameters across different developmental stages in the fly's life cycle. This study examined basic development markers, including fecundity (egg-laying), pupation height preference (larval habitat selection), eclosion (egg-to-fly development) and ovarian mitochondrial membrane potential. Briefly, the parent flies, as discussed in Section 2.3, were exposed to the treatment for 48 h. Subsequently, they were transferred to fresh vials every 24 h to assess fecundity by counting the eggs laid. Additionally, thirty eggs from each treatment vial were collected and placed in new vials to develop into adults. The number of eclosed flies was then counted to determine the egg-to-adult development ratio, expressed as a percentage [34]. Furthermore, twenty eggs laid were transferred to a new vial to estimate the pupation height preference, and the distance from the food surface to the pupae formation site was measured in millimetres [35].

2.6. Ovarian mitochondrial membrane potential analysis

The ovarian mitochondrial membrane potential was examined following an earlier reported method by Senthilkumar et al. [16]. To assess mitochondrial membrane potential, thirty female flies were exposed to control and treatment media, and their ovaries were subsequently dissected. The procedure was adapted from the Abcam TMRE-Mitochondrial Membrane Potential assay kit. Ovaries were dissected in Schneider's insect medium, incubated with 200 nM TMRE for 15 min in the dark, and then washed with PBS. The stained samples were examined under a Leica DM6 fluorescent microscope.

2.7. Statistical analysis

All experimental data are expressed as mean ± SEM unless otherwise specified. Statistical analysis was conducted using GraphPad Prism 6.0, with Dunnett's multiple comparison tests, to assess the significance of treatment groups against the control and ACR groups. A significance level of $p < 0.05$ was used.

3. Results

3.1. Standardisation of LC and MC concentrations

The treatment doses of LC and MC were determined experimentally based on standardization data presented in Fig. 1A to E. Base concentrations of 2.5, 5, 7.5, and 10 mg/ml were established, and survival and behavioural parameters were assessed to identify the optimal concentrations for both LC and MC. The control group (63 days; 65.07 mm/min; 99.62 %) and the solvent control (SC) group treated with glacial acetic acid (59 days; 61.18 mm/min; 98.88 %) served as baseline groups for chitosan dose standardization. Higher concentrations of LC and MC, particularly 7.5 mg/ml and 10 mg/ml, significantly impaired larval development, and locomotor activity. Among the LC treatments, a concentration-dependent decline in survival and behavioural outcomes was observed, with 2.5 mg/ml (61 days; 54.4 mm/min; 96.66 %) showing a non-toxic profile, exhibiting no significant changes in survival or behaviour compared to the control. In contrast, the 5 mg/ml (39 days; 48.4 mm/min; 85.92 %), 7.5 mg/ml (22 days; 77.25 %), and 10 mg/ml (10 days; 78.14 %) doses resulted in reduced survival and impaired behavioural and developmental outcomes.

Alternatively, comparing among MC groups, 5 mg/ml (49 days; 55.36 mm/min; 96.61 %) exhibited superior survival and locomotor abilities whereas 2.5 mg/ml (44 days; 51.99 mm/min; 86.7 %), 7.5 mg/ml (16 days; 95.55 %) and 10 mg/ml (11 days; 70.73 %) indicated significant reduction in locomotor activity rates and survival. According to these findings, low molecular weight chitosan (LC) at 2.5 mg/ml and medium molecular weight chitosan (MC) at 5 mg/ml were the best concentrations, with the least adverse effects and maximal protective advantages These concentrations demonstrated greater survival rates and locomotor activity than higher dosages, indicating their appropriateness for reducing acrylamide toxicity in the *Drosophila* model.

3.2. LC and MC variants restores ACR-induced survival and behavioural deficits

Fig. 2A to C illustrate the lifespan and locomotor parameters of fruit flies following five days of exposure to acrylamide (ACR) and the standardized chitosan variants (2.5 mg/ml LC and 5 mg/ml MC). The control (68 days; 68.62 mm/min; 95.55 %), SC (61 days; 67.14 mm/min; 94.44 %), 2.5 LC (64 days; 67.33 mm/min; 94.81 %) and 5 MC (50 days; 62.03 mm/min; 87.77 %) were established as the baseline groups for ACR toxicity and treatment efficacy analysis. Flies treated with ACR demonstrated early mortality with 11 days of survival capacity, a reduced crawling speed of 54.59 mm/min and a 78.14 % active adult fly population. However, the lethal effects of ACR were significantly reduced when co-exposed with chitosan variants. Co-treatment with ACR and standardised chitosan doses resulted in average lifespans and locomotor activity of 44 days; 60.18 mm/min; 92.96 % for ACR+LC (ALC) and 37 days; 58.7 mm/min; 86.66 % with groups exposed to ACR+MC (AMC).

Analysis of the beneficial effects of ALC and AMC revealed a significant increase in survival in the ALC group compared to the AMC group. Furthermore, ALC treatment demonstrated a 3 % improvement over AMC in larval crawling ability. In adult flies, the ALC group showed only a minimal 2 % reduction in climbing ability compared to controls, while AMC exhibited a more pronounced 9.3 % decrease, emphasising the superior efficacy of ALC in rescuing locomotor function following ACR exposure. Kumar et al., 2019 reported similar beneficial effects of marine-based LC against ACR-induced mortality and behavioural deficits in fruit flies [32]. However, the present study emphasises the protective effects between low and medium molecular weight chitosan, a concept which has not been extensively studied to the best of the authors' knowledge.

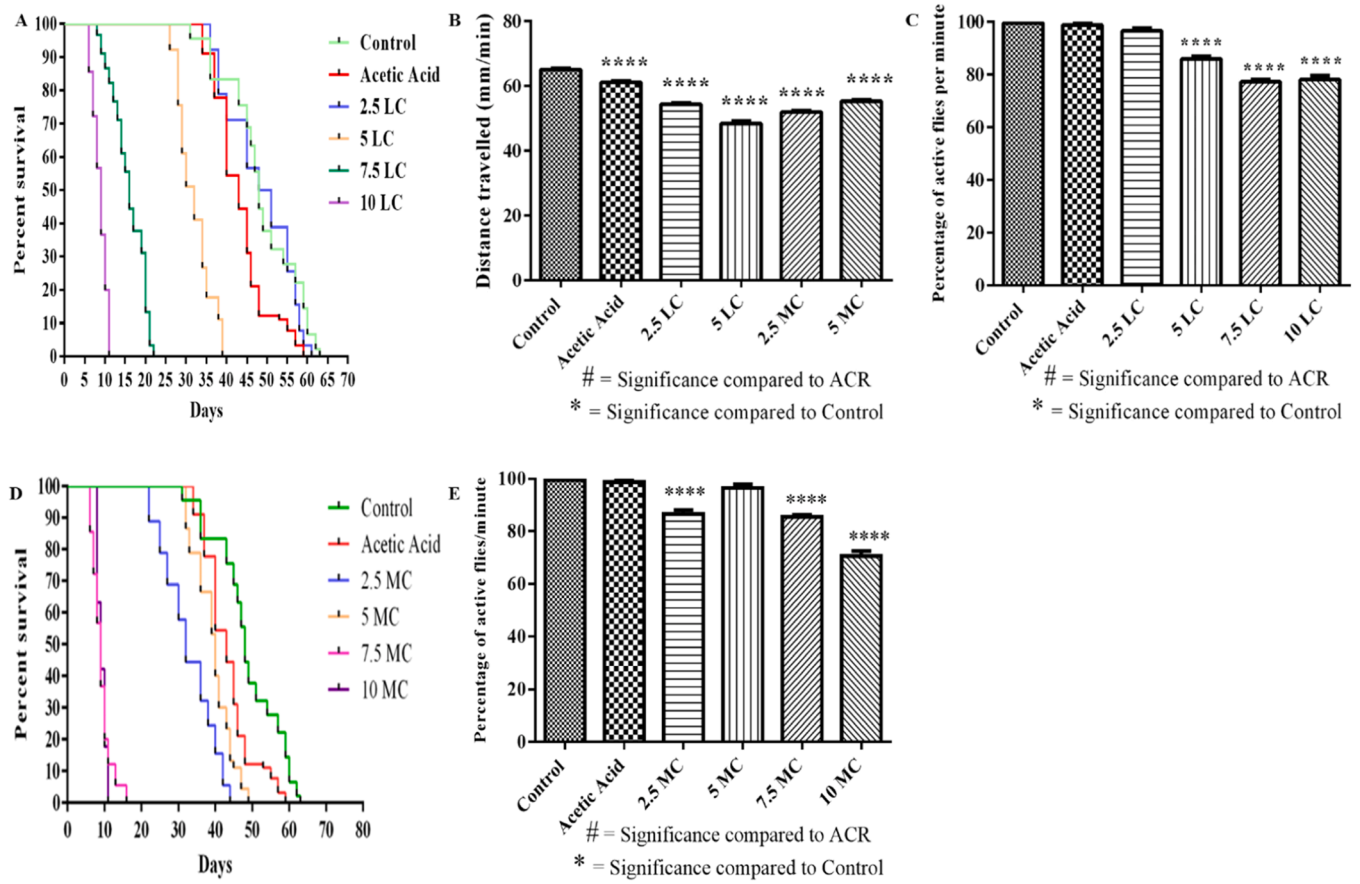

Fig. 1. Standardisation of LC and MC dose. Survival and locomotor functions of LC (A, B and C) and MC (C, D and E) treated larvae and flies (5 days exposure) were analysed to determine the effective concentration for further analysis.

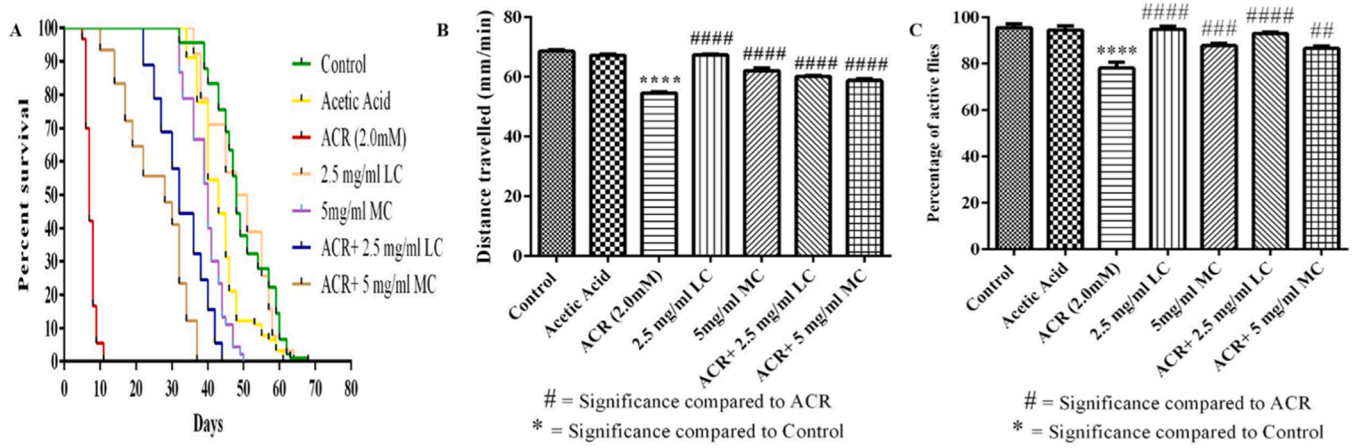

Fig. 2. Survival and Behavioural Analysis. Larvae and flies exposed to ACR and ACR+ Chitosan treatments for five days were analysed to determine the alterations in survival capacity (A), crawling function (B) and adult negative geotaxis behaviour (C).

3.3. Chitosan variants modulate ACR-induced biochemical deficits

The xenobiotic-induced alteration in an organism can be determined using alterations in stress markers like ROS levels and antioxidant enzyme (SOD) activities. As illustrated in Fig. 3A, ACR-treated flies demonstrated high DCF-DA intensity, a key indicator of increased ROS levels. Focusing on the baseline groups, SC and 2.5 LC did not exhibit any overt fluctuations in ROS levels however a minor peak in intensity was noted in 5MC treated flies compared to the control. Furthermore, the ALC and AMC groups exhibited subsequently lower ROS levels compared to the ACR group, whereas additional comparison between AMC and ALC highlighted the efficiency of ALC co-treatment against redox stress.

Further analysis of SOD activity as depicted in Fig. 3B showed ACR induced an increase in SOD activity levels compared to the baseline groups of control, SC and 2.5 LC. However, in alignment with the ROS data, 5 MC-treated flies demonstrated amplified SOD activity. Focusing on the co-treatment groups, ALC and AMC demonstrated significantly

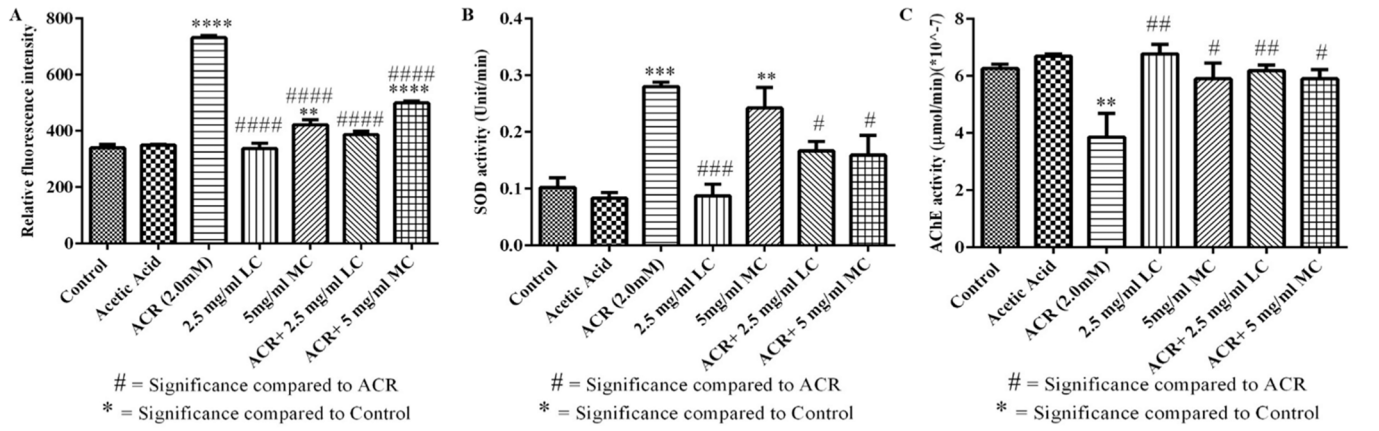

Fig. 3. Biochemical Parameters. Adult flies were exposed to respective treatments for five days and homogenised. The homogenates were used to estimate the levels of ROS (A) alongside the activities of SOD (B) and AChE enzymes (C).

lower activity rates compared to ACR further highlighting the antioxidant effects of chitosan variants. Yet, not much difference in activity was noted among the ALC and AMC groups. Additionally, this study also examined the activity of AChE, a vital enzyme that within the fly model can be used as a neurotoxic marker. AChE levels were significantly lower in ACR-treated flies compared to the baseline groups whereas, the co-treatment groups exhibited activity levels on par with the control group. Several studies have shown that ACR exposure negatively affects biochemical parameters such as ROS, SOD, and AChE levels. These abnormalities were normalized using antioxidants like curcumin, quercetin, and thymoquinone, which are known for their antioxidant properties [3,36–38]. In line with existing research, our study observed that LC co-treatment effectively maintained these biochemical levels at values similar to the control group, indicating minimal fluctuations despite ACR exposure. This suggests that LC is particularly effective in mitigating ACR-induced toxicity.

3.4. LC variant mitigates ACR-induced developmental deficits

The fecundity rate further confirmed the toxic effects of ACR, as shown in Fig. 4A. Treatment with 5 MC resulted in a 10.4 % decrease in fecundity, while 2.5 LC showed a smaller 4 % reduction compared to the control group. ACR exposure caused a significant 48 % reduction in fecundity. However, co-treatment with LC and MC improved fecundity, with the ALC group exhibiting a 75 % increase and the AMC group showing a 65 % increase, demonstrating the protective effects of chitosan treatments against ACR-induced toxicity.

The pupation height preference assay (Fig. 4B), which reflects habitat selection behaviour in third instar larvae, revealed that control and solvent control groups selected an average pupation height of 57 mm, while the ACR group chose a significantly lower height of 13.11 mm. The LC and MC groups showed average pupation heights of 56 mm and 58 mm, respectively. Notably, ALC treatment restored habitat selection behaviour, with larvae choosing an average height of 58.44 mm, while AMC-treated larvae preferred a lower height of 45.66 mm. Eclosion rates (Fig. 4C) demonstrated that control, solvent control, and LC groups achieved 100 % eclosion, while ACR and MC groups had reduced eclosion rates of 14 % and 80 %, respectively. ALC and AMC treatments improved eclosion rates to 93 % and 63 %, respectively, compared to the ACR group. Although AMC treatment showed some recovery, ALC treatment was the most effective in enhancing larval development and eclosion, demonstrating its superior protective effects.

Studies presenting evidence of ACR-induced developmental toxicity are limited in the existing literature and need more specific attention. However, the reproductive toxicity part of ACR has been established by a few studies that noted depletion of epididymal sperm reserves, weight differences in reproductive organs, and alteration in border cell migrations in rats and fruit flies, respectively [3,39,40]. However, we have demonstrated the effects of ACR and the therapeutic effects of chitosan as reproductive capacity evaluated by fecundity rate, pupation and adult emergence stages.

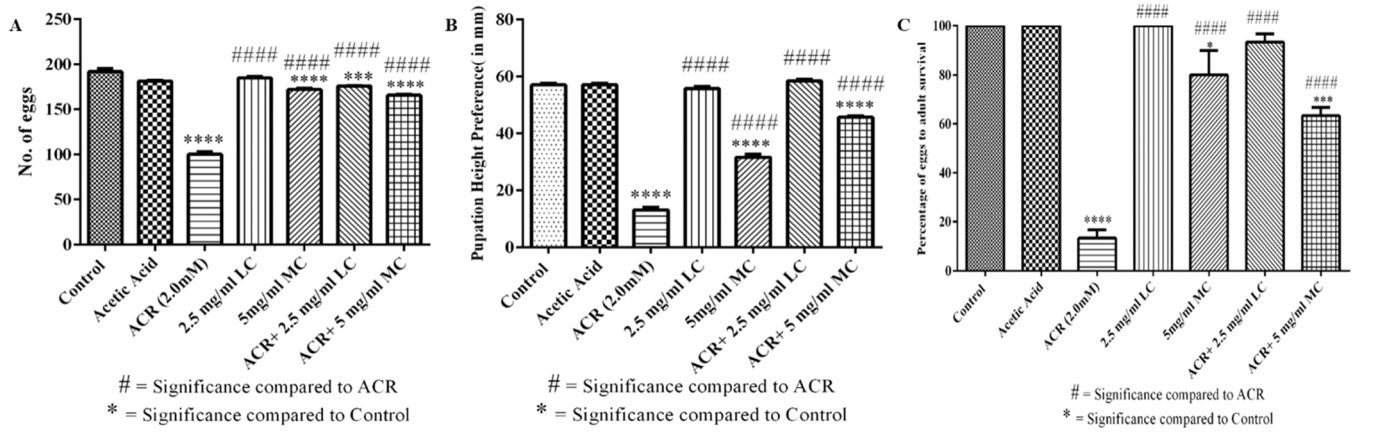

Fig. 4. Developmental Characteristics. Fruit flies exposed to respective treatment groups were utilised to investigate the influence of chitosan variants on ACR-induced developmental deficits through fecundity (A), pupation height preference (B) and eclosion rate (C).

3.5. LC variant normalised ACR-induced ovarian mitochondrial membrane potential

Tetramethyl rhodamine ethyl ester (TMRE) is a cell-permeant, positively charged red-orange fluorescent dye that accumulates in active mitochondria due to its relative negative charge. In healthy cells, TMRE accumulates in the mitochondria, emitting red fluorescence detectable by flow cytometry or fluorescence microscopy. However, in depolarized or inactive mitochondria, TMRE fails to accumulate, resulting in decreased fluorescence intensity. This property makes TMRE a valuable tool for assessing mitochondrial membrane potential in live cells [41].

Fig. 5 illustrates changes in mitochondrial membrane potential in control and treated ovary samples. ACR treatment caused a drastic 95.5 % decrease in TMRE fluorescence intensity, indicating mitochondrial membrane depolarisation. Chitosan co-treatments showed increased TMRE intensity compared to ACR, with ACR+LC demonstrating a 12 % increase and ACR+MC showing a 5 % increase. While both chitosan variants displayed significant reductions in fluorescence compared to the control, ACR+LC was more effective in recovering mitochondrial membrane depolarization. Additionally, dissected ovaries revealed that ACR-treated samples had lower TMRE intensity, reflecting increased mitochondrial depolarisation across various ovariole stages. In contrast, ACR+LC and ACR+MC treatments maintained more active mitochondrial populations, with ACR+LC showing a significantly lower depolarisation rate and better preservation of mitochondrial polarity in germarium cells compared to MC co-treatment.

Acrylamide (ACR) exposure has been shown to disrupt mitochondrial membrane potential (MMP) in *Drosophila melanogaster*, leading to mitochondrial dysfunction and associated cellular impairments [16]. In our study, we observed that ACR treatment resulted in a significant reduction in MMP, as evidenced by decreased fluorescence intensity in TMRE staining of fruit fly ovaries. This finding aligns with previous research indicating that ACR-induced toxicity can lead to mitochondrial depolarization and subsequent cellular damage. Conversely, co-treatment with low molecular weight chitosan (LC) effectively restored MMP to levels comparable to the control group, suggesting a protective role of LC against ACR-induced mitochondrial dysfunction. This observation is consistent with studies demonstrating that chitosan-coated probiotic nanoparticles can mitigate ACR-induced toxicity in *Drosophila*, including the restoration of mitochondrial activity [16].

4. Discussion

High-temperature processing produces byproducts like furans, nitrosamines, heterocyclic aromatic amines, and acrylamide (ACR), which are recognized as potential human carcinogens by regulatory bodies like the European Food Safety Authority (EFSA), the World Health Organization (WHO), and the US FDA. While permissible levels of these

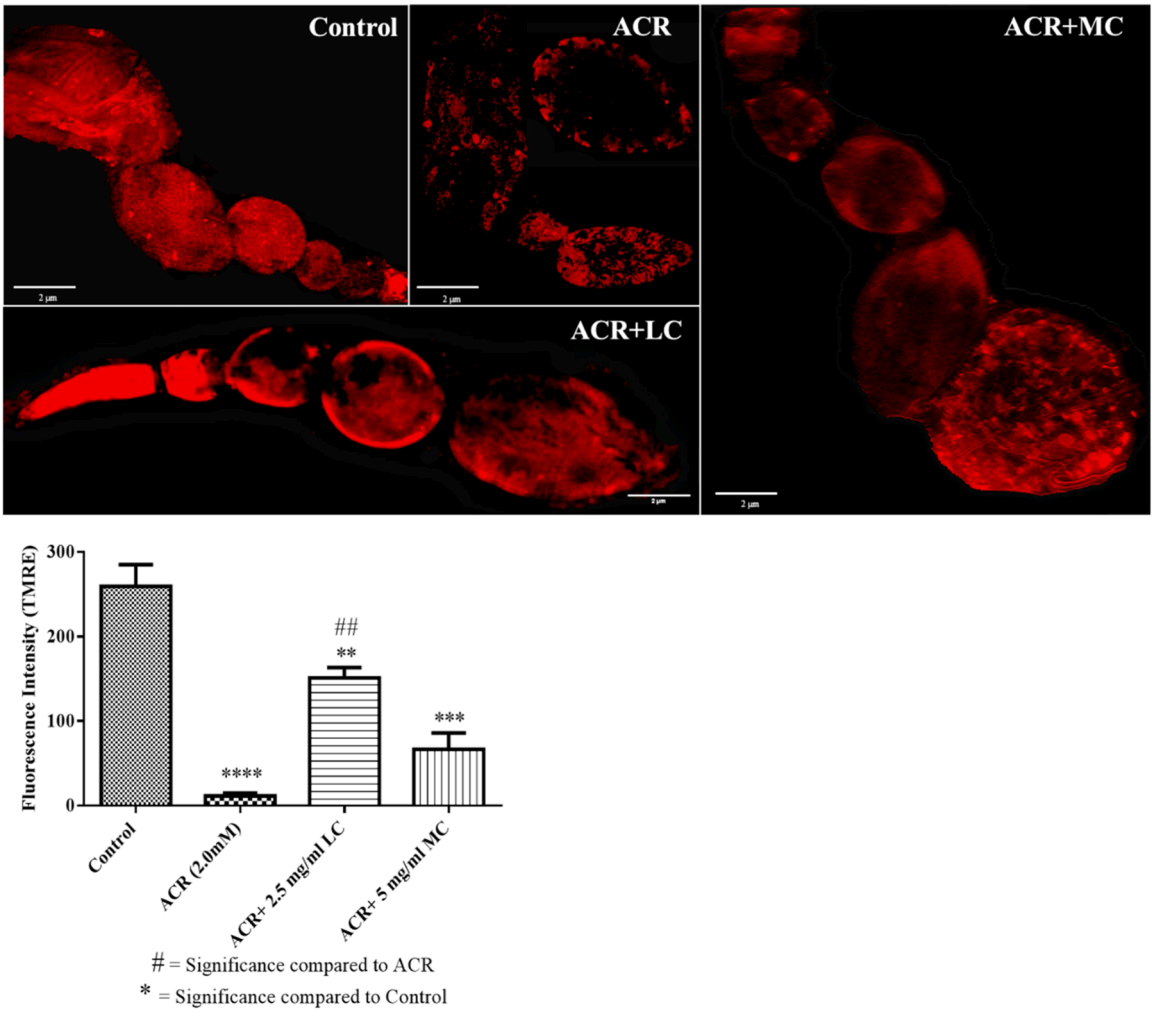

Fig. 5. Ovarian Mitochondrial Membrane Potential Estimation. TMRE staining of treated ovaries exhibited variations in fluorescence intensity between control, ACR and co-treatment groups. Increased TMRE intensity in control and ALC-treated ovaries indicates the presence of active mitochondrial pools.

contaminants are regulated for manufactured foods, the unregulated formation of heat-process toxins during home-based cooking remains a critical gap [42]. Acrylamide (ACR) is a neurotoxin with carcinogenic and genotoxic properties, but has a relatively small molecular weight and can easily be dispersed within the body. Studies indicate that acrylamide levels in foods range from 24 to 2942 μg/kg, with an average exposure of 0.4–1.9 μg/kg bw per day. The Joint FAO/WHO Expert Committee on Food Additives has highlighted the lack of information on acrylamide levels and formation in developing countries, which is crucial for developing strategies to reduce acrylamide concentrations in food [43].

Acrylamide, a genotoxic and carcinogenic substance, poses health risks due to its potential to damage genetic material and potentially cause cancer. Regulatory agencies have not established a tolerable daily intake (TDI) for acrylamide, as exposure could lead to measurable tumour incidence or other adverse effects. The EFSA established a Benchmark Dose Lower Confidence Limit (BMDL10) of 0.17 mg/kg body weight per day for tumour-related effects and 0.43 mg/kg body weight per day for neurological effects, signalling a public health concern at current exposure levels [3,44]. In this study, a 2 mM acrylamide concentration was used for fruit fly exposure, and based on the fly's daily food intake (220 ng of ACR/day) and body surface area, a rough estimate of a human equivalent dose was calculated as 0.35 mg/kg following the method reported in Mohideen et al. [45]. Although this estimate provides a general idea of dose standardization from fly to human, it is important to note that the actual translation of results requires more complex analysis.

The present study showed that ACR exposure caused early mortality, impaired neurobehavioral functions, and developmental deficits in fruit flies. This was accompanied by elevated redox stress markers, neurotoxic indicators, and mitochondrial depolarization in ovarian cells. However, co-treatment with chitosan variants mitigated ACR-induced toxicity, improving lifespans, normalizing behavioural patterns, and restoring developmental outcomes. The results also revealed differences in efficacy between low MW chitosan (LC) and chitosan-medium MW (MC) treatments, with LC showing superior protective capacity over MC in mitigating ACR-induced toxicity. These findings align with existing literature across diverse model systems [46–48]. The neurotoxic effects of ACR, along with behavioural and biochemical disruptions, have been well-documented [49–57]. Similarly, Pramod Kumar et al. (2020) demonstrated LMW chitosan's effectiveness against rotenone-induced Parkinson's disease, emphasizing its ability to cross biological barriers [58]. ACR's significant disruption of mitochondrial function, including its impact on redox balance, lipid metabolism, and apoptosis regulation, is corroborated by numerous studies [59,60].

Chitosan has been extensively used in the biomedical field, specifically as a drug-delivery system [12,13]. However, its antioxidative properties along with its prebiotic nature make it a superlative therapeutic option against process-based toxins within the food toxicology field. This can be evidenced by the beneficial effects reported by chitosan-coated probiotic nanoparticles against ACR-induced oxidative and mitochondrial stress in flies [16]. Additionally, the dose range of 2.5–10 mg/ml for chitosan variants used in this study was derived based on the neuroprotective effects of marine-based low MW chitosan against ACR reported in fly model [32]. Molecular weight plays a pivotal role in chitosan's biological activity and therapeutic efficacy against toxins like ACR. LMW chitosan, with its higher solubility and bioavailability, exhibits enhanced antioxidant and antimicrobial properties, facilitating superior mitigation of oxidative stress and cellular damage [61,62]. Its smaller molecular size supports better penetration into biological systems, promoting gut health and prebiotic effects [16,27,57]. Conversely, MMW and high molecular weight (HMW) chitosan, with lower solubility but higher viscosity, are better suited for structural applications, such as adsorbing larger molecules. However, their slower biodegradability and potential accumulation may limit therapeutic use [14,18,19]. These findings underscore the importance of molecular weight in optimizing chitosan's bioactivity, with LMW variants offering distinct advantages in applications requiring enhanced bioavailability and cellular interaction.

It is imperative to understand the difference between chitosan and other ACR mitigators at this junction. Various natural compounds and plant extracts have shown protective effects against acrylamide (ACR) toxicity across different models through mechanisms such as antioxidant activity, detoxification with glutathione (GSH), and inhibition of oxidative stress. For instance, *Acorus calamus*, *Panax ginseng*, *Zingiber officinale*, and *Allium sativum* improved motor behaviour, antioxidant enzyme activity, and lipid peroxidation in rodent models. Genistein, eugenol, and geraniol reduced oxidative stress, neuronal apoptosis, and locomotor deficits in both in vivo and in vitro systems, highlighting their anti-apoptotic and neuroprotective effects. Similarly, *Bacopa monnieri* and *Crocus sativus* restored oxidative balance and inhibited cellular damage, while pure phytochemicals like chrysin and epigallocatechin-3-gallate mitigated cytotoxicity and apoptosis in neuronal cell lines [63].

Additionally, few natural compounds and medicinal plants have demonstrated protective effects against acrylamide-related reproductive toxicity, genotoxicity, and general toxicity by leveraging mechanisms such as antioxidant activity, detoxification with glutathione (GSH), and inhibition of oxidative stress and inflammatory pathways. For reproductive toxicity, treatments like phenylethyl isothiocyanate and *Camellia sinensis* restored testicular histology and reduced spermatocyte damage by inhibiting CYP2E1 activity and enhancing antioxidant defences [63]. Genotoxicity mitigation was observed with *Aloysia triphylla*, which reduced DNA damage and increased plasma antioxidant capacity through polyphenol-mediated detoxification of acrylamide metabolites. Similarly, compounds like resveratrol and *Carica papaya* improved biochemical markers in multiple organs, indicating general systemic protection through GSH detoxification, free radical scavenging, and anti-inflammatory effects [63].

Against intestinal and hepatotoxicity, polyphenolic compounds like hydroxytyrosol, epicatechin, and myrcitrin effectively reduced oxidative stress, cell apoptosis, and DNA damage in cell and animal models. *Solanum fibers* improved intestinal epithelial integrity and reduced acrylamide absorption, while hepatoprotective agents like *Allium sativum*, *Digera muricata*, and allicin restored liver antioxidant enzyme activity and reduced lipid peroxidation and genotoxic markers [63]. The mitigators discussed primarily alleviate ACR toxicity by targeting oxidative stress through redox mechanisms. However, chitosan stands out not only for its antioxidant properties but also for its prebiotic nature, which modulates gut-mediated xenobiotic clearance pathways, offering comprehensive protection against ACR toxicity [5,14,16,49]. While this study did not delve deeply into the gut-mediated mechanisms of action, it establishes foundational evidence of chitosan's protective effects against ACR. These findings lay the groundwork for future research to explore the intricate interplay between chitosan's prebiotic effects and its role in enhancing xenobiotic metabolism via gut modulation.

Drosophila melanogaster is a valuable model organism for studying heat-processed toxin (HPT) toxicity due to its shared 75 % human disease-related genes, short life cycle, rapid generational turnover, cost-effectiveness, and well-characterized redox and detoxification systems. Its short life cycle, rapid generational turnover, and distinct developmental stages allow for high-throughput studies on developmental, reproductive, and lifespan effects, as well as neurobehavioral studies. However, translating findings from *Drosophila* to humans comes with limitations, primarily due to differences in metabolic pathways, enzymatic systems, and physiological structures. Additionally, variations in size, surface area, and metabolic rates between flies and humans complicate the standardization of doses. Therefore, the findings in *Drosophila* should be interpreted cautiously, with further validation needed through mammalian models or in vitro systems to assess the real-world implications of acrylamide exposure and its mitigation.

5. Conclusion and future research

This study highlights the protective potential of chitosan variants, particularly low molecular weight (LC), in mitigating acrylamide (ACR)-induced toxicity in *Drosophila melanogaster*. LC co-treatment effectively alleviated ACR-induced impairments in survival, behaviour, redox balance, mitochondrial function, fecundity, and eclosion rates, demonstrating its therapeutic promise. In contrast, medium molecular weight (MC) showed some recovery but was less effective, with the added drawback of inducing oxidative stress and developmental deficits. These findings highlight the importance of selecting the appropriate molecular weight of chitosan for optimal therapeutic efficacy, particularly for applications targeting heat-processed toxins. LC emerges as a potential candidate for future therapeutic strategies to mitigate the harmful effects of acrylamide and potentially other toxicants.

Future work should explore the role of gut-based mediation in chitosan's protective effects, with a focus on its potential as a prebiotic for therapeutic interventions against heat-processed food toxins. Additionally, more research is needed in the areas of reproductive and generational studies to better understand the long-term impact of ACR and chitosan treatment, particularly regarding its influence on developmental toxicity and its potential for improving offspring health. Moreover, the applicability of chitosan across different models of toxicity, as well as its role in mitigating the effects of other heat-processed food toxins, warrants further exploration. These studies could lay the groundwork for developing chitosan-based interventions for broader applications in toxicology and public health.

Ethical approval

Not Applicable.

CRediT authorship contribution statement

Swetha Senthil Kumar: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Software, Validation. **Sahabudeen Sheik Mohideen:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Swetha Senthil Kumar reports financial support and equipment, drugs, or supplies were provided by India Ministry of Science & Technology Department of Science and Technology. If there are other authors, they 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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Consent to participate

Not Applicable.

Consent to publish

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Data availability

Data will be made available on request.

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