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Protective Effects of L-Carnitine on Growth and Cardiac Function in High-Glucose-Exposed Zebrafish Embryos

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Abstract

Diabetes mellitus is a chronic metabolic disorder, and maternal hyperglycaemia during pregnancy increases the risk of fetal developmental complications. This study aimed to evaluate the protective role of L-carnitine against glucose-induced embryotoxicity in zebrafish (Danio rerio). Fertilized embryos were exposed to 5% glucose with or without L-carnitine supplementation (50 or 100 μM). Developmental outcomes including survival, hatching, malformations, body length, and heart rate were assessed using microscopy and analyzed by ANOVA and Kaplan–Meier survival curves. High glucose significantly reduced survival (hazard ratio = 6.86; $p < 0.0001$), delayed hatching (median hatching time 84 vs. 60 hpf; HR = 0.17, 95% CI = 0.08–0.39; $p < 0.0001$), and induced growth retardation and bradycardia ($p < 0.0001$). L-carnitine did not rescue survival or hatching but partially ameliorated growth impairment ($p < 0.05$) and restored heart rate in a dose-dependent manner, with 100 μM supplementation approaching control values ($p < 0.001$). Morphological abnormalities such as yolk sac edema and spinal curvature remained present but less severe with co-treatment. In conclusion, L-carnitine confers partial protective effects on growth and cardiac function under hyperglycaemic stress, although it does not prevent early lethality.

Keywords: Zebrafish model; Diabetes mellitus; Hyperglycaemia-induced embryotoxicity; L-carnitine supplementation.

Abbreviations: DM: Diabetes Mellitus; LC: L-Carnitine; LCLT: L-Carnitine L-tartrate; LCFA: Long-chain Fatty Acids; hpf: Hours Post Fertilization; YSE: Yolk Sac Edema; SC: Spinal Curvature; HR: Hazard Ratio; MST: Median Survival Time; MHT: Median Hatching Time; CI: Confidence Interval; bpm: Beats Per Minute

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronic hyperglycaemia, which remains a global health problems (International Diabetes Federation, 2021). The prevalence of hyperglycaemia during pregnancy is concerning, with epidemiological data showing that approximately 16% of live births from mothers aged 20–49 years are affected (Ogurtsova et al., 2017). Maternal hyperglycaemia not only increases the risk of obstetric complications but also imposes detrimental effects on fetal development, as infants of diabetic mothers are up to three times more likely to present with congenital abnormalities (Tinker et al., 2021). To date, preventive approaches to these complications have been limited to glycaemic control using antidiabetic medications, which may cause adverse effects for both the mother and foetus (Kalra et al., 2015). Thus, there is a clear need to explore safer complementary or adjunctive approaches to mitigate hyperglycaemia-induced embryotoxicity.

Zebrafish (Danio rerio) have emerged as a powerful vertebrate model for studying embryonic development and metabolic disorders. Zebrafish share 87% genetic homology and exhibit conserved mechanisms of glucose metabolism regulation with humans, making them well-suited for diabetes research (Capiotti et al., 2014; Chakraborty et al., 2016). Previous studies have demonstrated that high glucose exposure in zebrafish embryos leads to developmental abnormalities and reduced survival, thereby providing a reliable in vivo platform for investigating the consequences of hyperglycaemia (Li et al., 2022).

L-carnitine (LC) is a naturally occurring compound synthesized from lysine and methionine which can be obtained from dietary sources such as red meat, fish, poultry, and milk (Sawicka et al., 2020). LC plays a crucial role in the β-oxidation of long-chain fatty acids (LCFA) by facilitating the transport of fatty acids into mitochondria for metabolism. Additionally, LC has been shown to enhance non-oxidative glucose disposal in individuals with DM under hyperinsulinemic conditions

(Adeva-Andany et al., 2017). Importantly, experimental studies suggest that L-carnitine can reduce malformation rates, accelerate development, and improve larval survival rates of zebrafish embryos (Ma et al., 2020) .

Despite these promising findings, the potential of LC to counteract glucose-induced embryotoxicity remains to be fully elucidated. In particular, whether LC can mitigate hyperglycaemia-associated defects in zebrafish embryos remains unknown. To address this gap, the present study was designed to investigate the protective role of LC against glucotoxicity in zebrafish embryos exposed to high glucose. Specifically, we evaluated the effects of LC on embryo survival, hatching rate, developmental malformation, body length, and heart rate as key endpoints.

MATERIAL AND METHODS

Materials

D-glucose was obtained from Himedia (Lot no. 0000522073, Maharashtra, India). L-carnitine in the form of L-carnitine tartrate (LCLT) was purchased from Norvine (Georgia, USA). All other chemicals used were of analytical grade.

Zebrafish maintenance and embryo collection

All husbandry and experimental procedures were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, Indonesia (No.98/EC/KEPK/04/2025). Adult wild-type zebrafish (Danio rerio) were maintained at the Laboratory of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya (Malang, Indonesia). Zebrafish were housed at 28 ± 0.5 °C under a 14 h light/10 h dark cycle. For spawning, male and female zebrafish were separated overnight in screened tanks and released together in the morning, as previously described (Thompson et al., 2024; Yoon et al., 2024). Freshly fertilized eggs were collected in Petri dishes containing E3 embryonic medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, and 1× methylene blue) and subsequently transferred to culture plates for further treatment.

Embryotoxicity study

An initial toxicity test was conducted to determine optimal glucose concentration and non-toxic LC dose ranges. Fertilized eggs (~2 h post fertilization, hpf) were grouped into 6-well plates (two replicate per treatment group), each replicate consisted of five embryos. The control group was maintained in 3 mL of E3 medium. Glucose-exposed groups were treated with 2%, 5%, or 10% D-glucose in E3, whereas LC groups were treated with 10, 50, or 100 µM LCLT dissolved in E3. Embryos were incubated at 28 °C under a 14 h light/10 h dark cycle until 144 hpf. Mortality and hatching were recorded at 12 h intervals, while the medium was refreshed every 24 h. For the second series of experiment, we evaluated the effect of LC supplementation in addition to high glucose treatment in zebrafish embryo. The key developmental endpoints observed were survival, hatching rate, developmental malformation, body length, and heart rate.

Embryo Survivability Evaluation

The zebrafish larvae were raised until death. Early embryonic death was identified by loss of translucency and changes in coloration, while larval death was defined by the absence of heartbeat(Westerfield, M., 2007). Any dead embryo or larvae were discarded promptly. Cumulative survival was analyzed and presented as Kaplan–Meier survival plots.

Embryo Hatchability Evaluation

Embryo hatching, which typically occurs between 48–72 hpf, was assessed visually at 12 h intervals. Newly hatched larvae were counted, and cumulative hatching was expressed as the percentage of embryos hatched at each time point.

Embryo Heart Rate Measurement

Larval heart rate was measured at 72 hpf. Individual larvae were positioned laterally on glass slides and observed under a Nikon Eclipse Si binocular microscope (Japan) at 40× magnification. Heartbeats were counted visually for 10 s and converted to beats per minute (bpm). Measurements were repeated at least three times per treatment group to obtain average values.

Larval Body Length Measurement

Larval body length was measured at 72 hpf. Larvae were laid on lateral position microscope slides and photographed under a microscope. Body length was defined as the distance from the anterior tip of the head to the end of the tail, following the spinal axis, and measured using imageJ analysis software (Hong et al., 2024). Ten larvae per treatment group were analyzed, and mean body length was calculated.

Larval Malformation Observation

Larvae were visually examined at 24 h intervals under a Nikon Eclipse Si microscope (40×) for morphological malformations. Typical abnormalities recorded included pericardial edema, yolk sac edema, spinal curvature, and craniofacial or tail deformities (Ma et al., 2020).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and analyzed using GraphPad Prism version 9.0 (Boston, USA). Differences among groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. To evaluate the probability of survival and hatching in each treatment group, time-to-event data were analyzed using log-rank tests and

presented as Kaplan–Meier survival curves with 95% confidence intervals (CI). A p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of different glucose and LC concentrations on the survival rate and hatching rate of zebrafish embryos

Kaplan–Meier survival analysis (Figure 1) demonstrated that zebrafish embryos maintained in standard embryonic medium exhibited high survivability for up to 144 hpf. The log-rank test for trend analysis revealed significant difference between groups (p value=0.0363) (Figure 1A). Exposure to increasing glucose concentrations led to a dose-dependent reduction in survival, with embryos in 10% glucose showing complete lethality by 60 hpf (median survival time=42 h), and those in 2% and 5% glucose by 96 hpf (median survival time=72 h). In contrast, treatment with LC (50–100 µM) did not differ significantly from control. Interestingly, treatment with 10 µM LC caused significant decrease in cumulative survival (log-rank test, p value=0.0076).

Additionally, glucose impaired zebrafish embryo hatching in a dose-dependent manner, with 10% glucose nearly abolishing hatching altogether. Meanwhile, treatment with LC (10–100 µM) did not interfere with hatching, as LC-treated embryos reached hatching rates comparable to controls (median hatching time=60 h) (Figure 1B). For these reasons, LC at 50 and 100 µM were chosen for the subsequent trial.

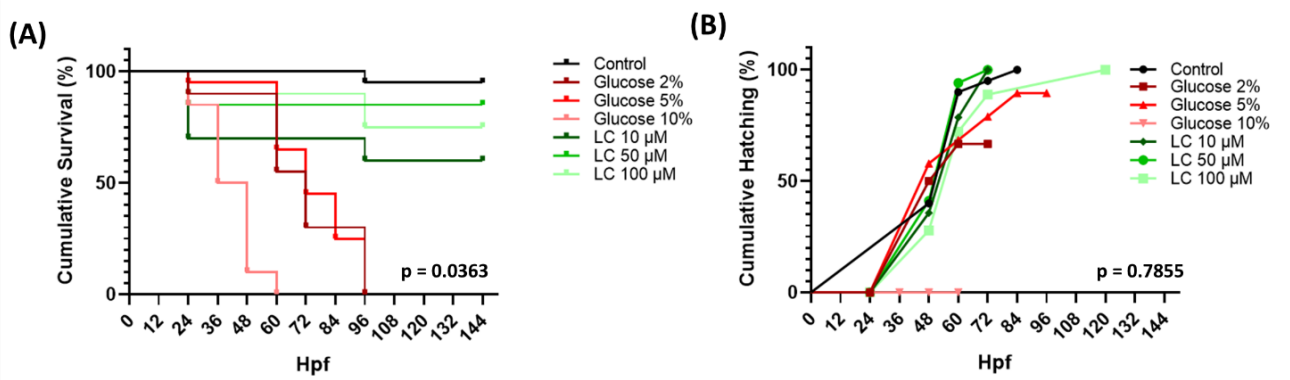
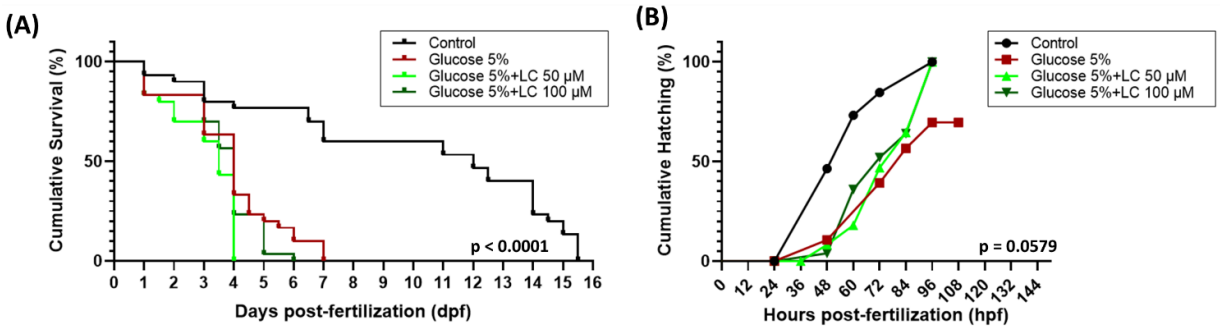


Figure 1. Effect of glucose and L-carnitine on zebrafish embryo survival and hatching. (A) Kaplan–Meier survival curves and (B) Cumulative hatching rates of zebrafish embryos exposed to different concentrations of glucose (2–10%) or L-carnitine (10–100 µM). Data are presented as the percentage of embryos reaching the respective endpoint (death or hatched), obtained from two independent replicates (n=20 embryos per group).

L-Carnitine effect in high-glucose–exposed zebrafish embryos survivability and hatchability

To evaluate whether L-carnitine (LC) supplementation could mitigate glucose-induced embryotoxicity, zebrafish embryos were exposed to 5% glucose with or without LC (50 or 100 µM). As shown in the Kaplan Meier plot (Figure 2A), exposure to 5% glucose markedly reduced survival compared to controls (HR = 6.86, p < 0.0001). Surprisingly, supplementation with 50 µM LC slightly worsened survival (HR = 2.65, p = 0.0363), while 100 µM LC did not significantly affect survival compared with glucose alone.

Similarly, cumulative hatching analysis revealed that 5% glucose delayed embryo hatching compared to controls (median hatching time [MHT] = 84 vs. 60 hpf; HR = 0.17, 95% CI = 0.08–0.39, p < 0.0001). However, LC supplementation at either 50 or 100 µM failed to significantly improve hatching rates (Figure 2B). These results indicate that LC supplementation at the tested concentrations did not rescue the reduced survival or impaired hatching induced by high-glucose exposure.



Group	MST (dpf)	HR	95% CI	P value
Control vs Glucose				
Control	12	-	-	-
Glucose 5%	4	6.86	3.36 to 13.97	<0.0001
Glucose vs LC				
Glucose 5%	4	-	-	-
Glucose 5%+LC 50 µM	3.5	2.65	1.31 to 5.36	0.0363
Glucose 5%+LC 100 µM	4	1.46	0.77 to 2.78	ns

Group	MHT (hpf)	HR	95% CI	P value
Control vs Glucose				
Control	60	-	-	-
Glucose 5%	84	0.17	0.08 to 0.39	<0.0001
Glucose vs LC				
Glucose 5%	84	-	-	-
Glucose 5%+LC 50 µM	84	1.94	0.88 to 4.28	ns
Glucose 5%+LC 100 µM	72	2.08	0.98 to 4.42	ns

Figure 2. Effect of L-carnitine supplementation on survival and hatching of zebrafish embryos exposed to high glucose. (A) Kaplan–Meier survival curves and (B) cumulative hatching rates of embryos (Figure 2) exposed to 5% glucose with or without L-carnitine (50 or 100 µM), compared with controls. Data are expressed as percentages of embryos reaching the respective endpoint, with statistical analysis performed using log-rank tests (n = 30 embryos per group, two independent replicates). HR: Hazard Ratio; MST: median survival time; MHT: median hatching time.

Effect of glucose and L-carnitine on zebrafish larval body length

To assess the impact of glucose and LC treatment on zebrafish somatic growth, larval body length was measured at 72 hpf (Figure 3). Embryos exposed to 5% glucose exhibited a significant reduction in body length compared to controls ($p < 0.0001$). Supplementation with both 50 µM and 100 µM L-carnitine partially attenuated the stunted growth, resulting in significantly longer larvae compared to the 5% glucose group ($p < 0.05$).

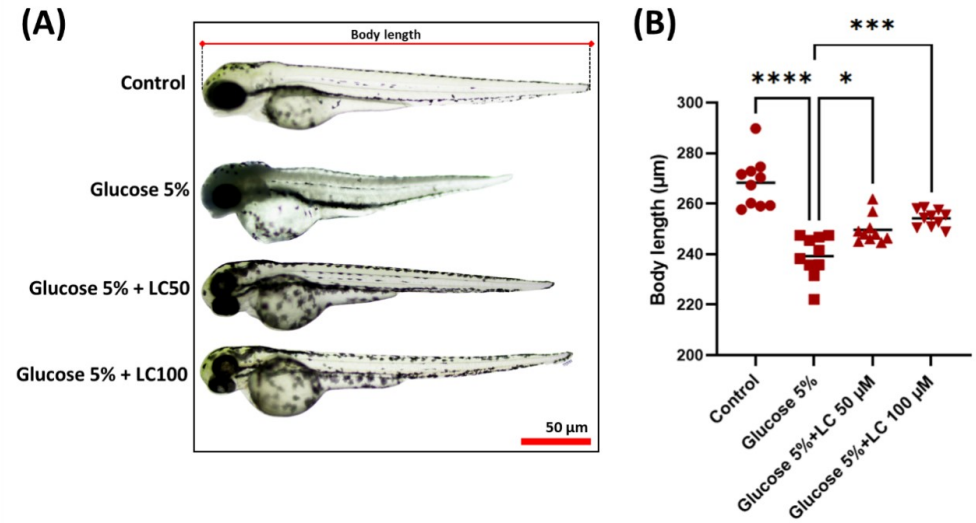


Figure 3. Effect of glucose and L-carnitine on zebrafish larval body length at 72 hpf. Picture was taken at 40x. Data are presented as mean ± SD (n = 10 larvae per group). Statistical significance was determined using one-way ANOVA followed by post hoc. ****$p < 0.0001$; ***$p < 0.001$; *$p < 0.05$. Scale bar = 50 µm.

Effect of glucose and L-carnitine on zebrafish larval heart rate

To determine the impact of glucose and L-carnitine on cardiac function, heart rate was assessed in zebrafish larvae at 72 hpf. Heartbeats were counted over 10-second intervals under a microscope and extrapolated to beats per minute (bpm) (Figure 4). Exposure to 5% glucose resulted in a significant reduction in heart rate compared to controls ($p < 0.0001$). Co-treatment with 50 µM L-carnitine resulted in a modest but significant increase in heart rate compared to the glucose group ($p < 0.05$). Notably, supplementation with 100 µM L-carnitine restored heart rate close to control levels ($p < 0.001$). These results indicate that high glucose impairs cardiac function and L-carnitine supplementation exhibited a dose-dependent protective effect.

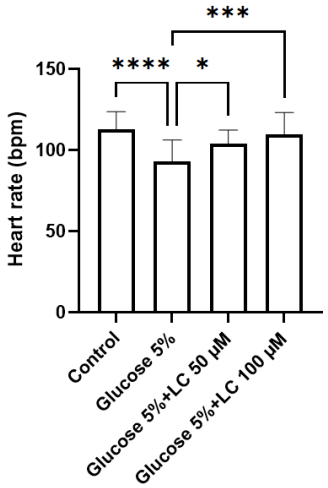


Figure 4. Effect of glucose and L-carnitine on zebrafish heartbeat rate at 72 hpf. Data are presented as mean with SD (n = 18 larvae per group). Statistical significance was evaluated using one-way ANOVA followed by post hoc Tukey. ****p < 0.0001; ***p < 0.001; *p < 0.05.

Effect of glucose and L-carnitine on morphological malformations in zebrafish embryos

Morphological observation (Figure 5) revealed that embryos in the control group developed normally without visible malformations at both 72 and 96 hpf. In contrast, exposure to 5% glucose induced multiple abnormalities, including yolk sac edema (YSE), spinal curvature (SC), and hyperemia (HY) (Figure 5). Co-treatment with LC did not entirely prevent these defects, as YSE and HY were still evident. However, supplementation with LC reduced the severity of malformations, with less severe spinal curvature and edema compared to glucose alone.

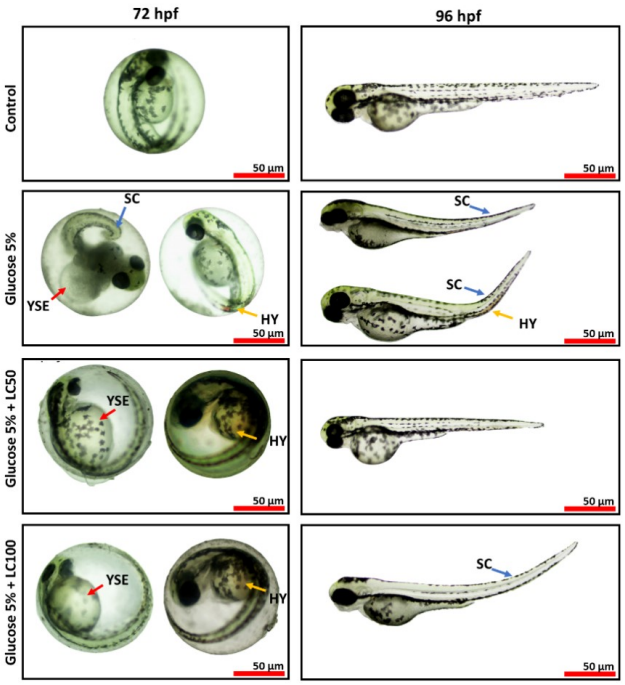


Figure 5. Representative images of zebrafish embryos and larvae at 72 and 96 hpf under different treatment conditions (Magnification 40x). YSE: yolk sac edema, SC: spinal curvature, HY: hyperemia. Scale bar = 50 µm.

Discussion

In this study, we demonstrated that high glucose exposure induces marked developmental toxicity in zebrafish embryos, characterized by reduced survival, impaired hatching, morphological malformation, stunted growth, and bradycardia. A glucose concentration of 5% was employed in this study to simulate hyperglycemia. Although this concentration is approximately 20-fold higher than the hyperglycaemic level observed in diabetic patients, it has been reported to increase free glucose level to ~19 mmol/l (342 mg/dl) comparable to those found in uncontrolled diabetic patients (Singh et al., 2019; Thompson et al., 2024).

Exposure to glucose decreased survival and hatching rates in a dose-dependent manner, with complete lethality observed at a 10% glucose concentrationby 60 hpf. In the present study, 5% glucose exposure resulted in 80% of larval mortality by 120 hpf (median survival of 96 hpf), which is significantly higher than prior reports showing only ~37.5% mortality at the same time point (Singh et al., 2019). Meanwhile, other study reported that 2.5% glucose exposure caused ~80% mortality rate at 120 hpf (Li et al., 2022). Such discrepancies may reflect strain- or stock-related differences in susceptibility despite using the same species. Nonetheless, our results corroborate the well-established concept that hyperglycemia is highly detrimental to embryonic and larval viability, as it is known to increase oxidative stress, metabolic disturbances, apoptosis, and impairs normal development in zebrafish which significantly reduce the survival rate(Li et al., 2022; Thompson et al., 2024). Consistent with earlier findings, hyperglycemia also induced morphological abnormalities, including yolk sac edema, spinal curvature, and hyperemia. Transcriptomic analyses further support these phenotypes, showing demonstrating that high glucose downregulates developmental signaling pathways including Wnt, Notch, FoxO, PPAR, and p53 (Thompson et al., 2024).

To our knowledge, studies evaluating the toxicity of LC in zebrafish embryo are scarce. In our study, LC at 50–100 µM was well tolerated, but did not affect survival or hatching, while 10 µM unexpectedly reduced survival. This contrasts with earlier findings, where at dose as low as 1 mg/L (~6.2 µM), LC promoted early hatching and improved survival compared to control (Ma et al., 2020). However, in Mat et al. study, the control group did not start hatching until 72 hpf, while in our study the embryos began hatching at 48 hpf. Interestingly, the control group and LC at 0.5 mg/l in that study also showed relatively low survival rate, less than 50% at 60 hpf, while control group in our study maintain at 90% survival rate in the same time point. This may contribute to the overestimation of protective effect of LC supplementation in zebrafish embryo. Additional evidence suggests that carnitine may exert biphasic or threshold-dependent effects: for example, acetyl-L-carnitine (ALCAR) at 1 mM increased ATP and ROS

production in zebrafish larvae(Gu et al., 2021), a dose nearly 10-fold higher than our maximum concentration. However, that study did not report the mortality rate of the treatment group, despite stating that ALCAR did not pose any toxicity to zebrafish embryo. These findings suggest that both insufficient and excessive supplementation can disrupt metabolic balance, leading to adverse outcomes.

In contrast to early-stage endpoints, our study results indicated that LC supplementation partially ameliorated later developmental endpoints. A significant reduction in heart rate was observed in glucose-treated larvae, supporting the notion that hyperglycaemia compromises cardiac development and function. Co-treatment with LC restored heart rate in a dose-dependent manner, with 100 μM LC nearly normalizing the heart rate to the control levels. This cardioprotective effect is consistent with LC's established role in enhancing fatty acid utilization and mitochondrial ATP production, which are critical for sustaining cardiac contractility given the myocardium's dependence on fatty acids as its primary energy substrate (Longnus et al., 2001; Ma et al., 2020; Schulz, 1994). Although some studies reported no effect of LC on zebrafish embryonic heart rate under short-term exposures (2-20h) (Gu et al., 2021; Kanungo et al., 2012), the longer treatment in our model may have been necessary to reveal its benefit under metabolic stress.

Similarly, LC supplementation attenuated growth impairment and reduced the severity of morphological abnormalities caused by high glucose exposure. These findings are consistent with previous reports, which show that LC improves body length and reduces deformity rates in zebrafish larvae (Ma et al., 2020). Mechanistically, these effects likely reflect LC's role in sustaining mitochondrial β-oxidation, reducing oxidative stress, and facilitating the removal of toxic metabolic byproducts, thereby maintaining the supply of energy for highly-demanding physiological processes such as growth and cardiac function, which are often compromised during glucotoxic stress (Virmani & Cirulli, 2022).

In summary, our findings demonstrate that while L-carnitine did not improve survival or hatching under high-glucose conditions, it conferred measurable protective effects on larval growth and cardiac function. This suggests that LC may be more effective in somatic growth and cardiac activity, rather than preventing early lethality caused by severe glucotoxic stress. Further studies are warranted to optimize dosing strategies, explore synergistic antioxidant interventions, and investigate molecular mechanisms such as β-oxidation, IGF-1 signaling, and ROS scavenging that may underlie LC's partial protective effects.

CONCLUSIONS

LC supplementation at 50–100 μM was well tolerated, partially attenuating growth retardation and restoring heart rate in a dose-dependent manner. However, LC did not rescue survival, hatching rates, or morphological deformity under hyperglycaemic conditions, indicating that its protective capacity may be limited against severe glucotoxic stress. These findings suggest that LC has potential as a complementary agent to mitigate selected developmental complications of hyperglycaemia, but further studies are required to optimize dosing and elucidate underlying molecular mechanisms.

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Authors' Contributions: EF conceived and designed the study, supervised the experimental work, and finalized the manuscript. AW contributed to methodology development, performed data collection, and participated in data analysis. LWS assisted in the experimental procedures, data interpretation, and manuscript drafting. NDP assisted in the research process and contributed to the preparation and submission of the manuscript. PKC assisted in the research process, figure preparation, and manuscript revision. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

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