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RESEARCH **Open Access**

Nanoemulsion of *Curcuma longa* Extract Improves the Developmental Process in High Glucose-Induced Zebrafish (*Danio rerio*) Embryos

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Abstract
Gestational diabetes mellitus (GDM) is hyperglycemia during pregnancy, which can increase oxidative stress and reactive oxygen species (ROS). This oxidative stress can adversely affect fetal developmental processes, including body length, ocular development, blood flow, and heart rate. *Curcuma longa* extract, containing curcumin, has been shown to possess antidiabetic and antioxidant properties. Nanoemulsions have demonstrated efficacy in enhancing the pharmacokinetics and pharmacodynamics of herbal compounds. This study aimed to evaluate the effect of a nanoemulsion of *Curcuma longa* extract on body length, ocular apoptosis, blood flow, and heart rate in zebrafish (*Danio rerio*) embryos exposed to high glucose concentrations. Zebrafish has many benefits as it has 70% orthologous genes with humans, high fecundity, and rapid embryogenesis. Nanoemulsions were prepared using the self-nanoemulsifying drug delivery system (SNEDDS) technique. Embryos were allocated to five groups: control, 3% glucose, and 3% glucose with 1.25, 2.5, or 5 µg/mL nanoemulsion of *Curcuma longa* extract. The results showed that a 1.25 µg/mL nanoemulsion of *Curcuma longa* extract reduced phosphoenolpyruvate carboxykinase (PEPCK) expression, increased body length, and mitigated developmental process disorders by decreasing ocular apoptosis and maintaining blood flow and heart rate in high glucose-induced zebrafish embryos. These findings suggest that a nanoemulsion of *Curcuma longa* extract may have potential therapeutic applications in managing the adverse effects of hyperglycemia on fetal development in GDM.

Keywords: *Curcuma longa*, Nanoemulsion, GDM, Hyperglycemia, PEPCK, Development, Zebrafish.

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1. INTRODUCTION

Hyperglycemia affects 16.7% of all pregnancies worldwide, of which 80.3% are caused by gestational diabetes mellitus (GDM) (International Diabetes Federation, 2021). It has been reported that 25.9% of Southeast Asian women have GDM (Al-Biltagi et al., 2021). In Indonesia, the prevalence of GDM ranges from 1.9-3.6% (Safitri et al., 2022). This variation in prevalence is dependent on population characteristics, genetic factors, and the diagnostic criteria used during screening (Nguyen et al., 2018). However, there is a global trend for the prevalence of GDM to increase in parallel with advanced maternal age, obesity, and Diabetes Mellitus Type 2 (DMT2) levels (Nijs & Benhalima, 2020).

During pregnancy, women undergo physiological changes that produce insulin-antagonizing hormones, such as progesterone, estrogen, human placental lactogen, and cortisol. These hormones increase insulin resistance. When pancreatic β-cells are unable to compensate for insulin resistance, hyperglycemia can occur, triggering an increase in reactive oxygen species (ROS) (Giussani, 2016; Kumar et al., 2015). Increased ROS production stimulates oxidative stress. Several fetal organs such as the kidneys, nerves, blood vessels, and eyes are damaged by oxidative stress (Kumar et al., 2015). Damaged blood vessels can cause chronic hypoxia due to the difficulty of oxygen entering the blood, especially placental blood flow to the fetus. This condition can also result in a decreased heart rate and delayed fetal growth (Giussani, 2016).

Among potential therapeutic agents, *Curcuma longa* extract is known to exert antidiabetic effects (Chuengsamarn et al., 2012). Curcumin is a primary compound involved in this process. Curcumin contains antioxidants that balance free radicals and trigger an increase in insulin production by suppressing pancreatic cell damage (Pratiwi et al., 2016). Additionally, *Curcuma longa* increased insulin sensitivity. Curcumin in *Curcuma longa* can accelerate insulin response, thereby reducing blood glucose levels. Although its antidiabetic effects are well known, the absorption rate of curcumin is low. Therefore, the development of herbal formulations using the nanoemulsion method is necessary. Nanoemulsions have been shown to increase the efficacy and absorption of herbs, thereby enhancing their antidiabetic effects (Naseema et al., 2021). Nanoemulsions have the advantage of being able to encapsulate herbal bioactive compounds, thereby increasing their stability, producing small droplets, and a larger surface area, thus providing greater absorption, reducing doses, and providing better therapeutic effects (Harwansh et al., 2019; Jaiswal et al., 2015).

Zebrafish (*Danio rerio*) have been extensively used as experimental animals for the development of metabolic diseases. Zebrafish possess 70% genes orthologous to humans, high fecundity, and rapid embryogenesis (Jeevanandam et al., 2019; Zakaria et al., 2018). Furthermore, zebrafish embryos aged 0-72 hours post-fertilization (hpf) are equivalent to fetuses (Khotimah et al., 2021). Previous research by Singh et al. (2019) showed that induction of zebrafish embryos at high glucose concentrations resulted in hyperglycemia through increased expression of phosphoenolpyruvate carboxykinase (PEPCK). PEPCK catalyzes gluconeogenesis, which converts oxaloacetate to phosphoenolpyruvate. This demonstrated that PEPCK is a representative marker of changes in glucose metabolism (Khotimah et al., 2022; Salehpour et al., 2021). There is no research that examines the effect of *Curcuma longa* nanoemulsion on the growth of zebrafish embryos exposed to high glucose. This study aimed to evaluate the effect of a nanoemulsion of *Curcuma longa* extract on body length, apoptosis in the eye, blood flow, and heart rate in zebrafish embryos induced by high glucose.

2. RESEARCH METHOD

Fertilized zebrafish embryos (*Danio rerio*) 0-2 hpf (hour post-fertilization) from the Fish Cultivation Laboratory Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, were selected based on the criteria of still having chorion, a clear, transparent color, and no fungus when viewed with a microscope connected to the software Optilab. The samples were collected

at random, with 60 zebrafish embryos per group. To prepare embryonic medium, stock solutions ware made with 0.25 grams of CaCl, 0.15 grams of KCl, 5 grams of NaCl, and 0.815 grams of MgSO4. The stock solution was then diluted in 500 mL distilled water to a final concentration of 10 times (Khotimah et al., 2021). Before use, the stock solution was dissolved in aqueous solution at a ratio of 1:9. *Curcuma longa* extract was obtained and certified from UPT Materia Medica Batu, Malang, East Java, Indonesia. Using the self-nanoemulsifying drug delivery system (SNEDDS) technique, soybean oil, PEG 40, Span 80, and PEG 400 in ratios of 16.37, 32.07, 11.56, and 40% were used to create nanoemulsions (Puspita et al., 2016). A zeta potential and particle size analyzer (PSA) at the Institut Teknologi Sepuluh Nopember Surabaya (ITS) was used for the measurement of nanoparticles < 100 nm. A 3% glucose solution was prepared using 30 g of glucose powder (Merck, 108337, Germany) and dissolved in 100 ml of aquadest. Before use, the stock solution was dissolved in an embryonic medium at a ratio of 1:9. In this study, the treatment groups were divided into five groups (Table 1):

Table 1. Treatment Group

Group	**Treatment**
Control (C)	Given 5 mL solution containing embryonic medium in each well
Glucose (G)	Given 5 mL solution containing embryonic medium and 3% glucose in each well
Treatment group 1 (T1)	Given 5 mL solution containing embryonic medium, 3% glucose, and 1.25 µg/mL nanoemulsion of *Curcuma longa* extract in each well
Treatment group 2 (T2)	Given 5 mL solution containing embryonic medium, 3% glucose, and 2.5 µg/mL nanoemulsion of *Curcuma longa* extract in each well
Treatment group 3 (T3)	Given 5 mL solution containing embryonic medium, 3% glucose, and 5 µg/mL nanoemulsion of *Curcuma longa* extract in each well

All treatments were administered from 2 hpf to 3 days post-fertilization (dpf) and replaced every 24 h. One-way analysis of variance (ANOVA) was used for statistical data analysis using IBM SPSS software version 25. This study has been authorized by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Brawijaya (ethics number No.137/EC/KEPK/05/2022).

Reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure PEPCK expression. (Khotimah et al., 2022). RNA isolation and purification were performed on five sample groups of 72 hpf zebrafish larvae using the Tissue Total RNA Mini Kit (Geneaid, catalog number RT100). cDNA synthesis was performed according to the ReverTra Ace qPCR RT Master Mix protocol with a gDNA Remover kit (TOYOBO, catalog number FSQ-301). The primer sequences were as follows: PEPCK forward, 5'-GAGAATTCTCACAC ACACACACGTGAGCA GTA-3'; PEPCK reverse, 5'-GTAAAAGCTTTCCGCCA TAACATCTCCAGCA GAA-3'. The PCR conditions (PCR Bio Rad T100 Thermal Cycler) were 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min. The results were visualized by electrophoresis using a Gel Doc EZ Imager and quantified using a Bio-Rad Gel Doc Imaging System. As an internal control, the gene expression level was matched to that of β-actin.

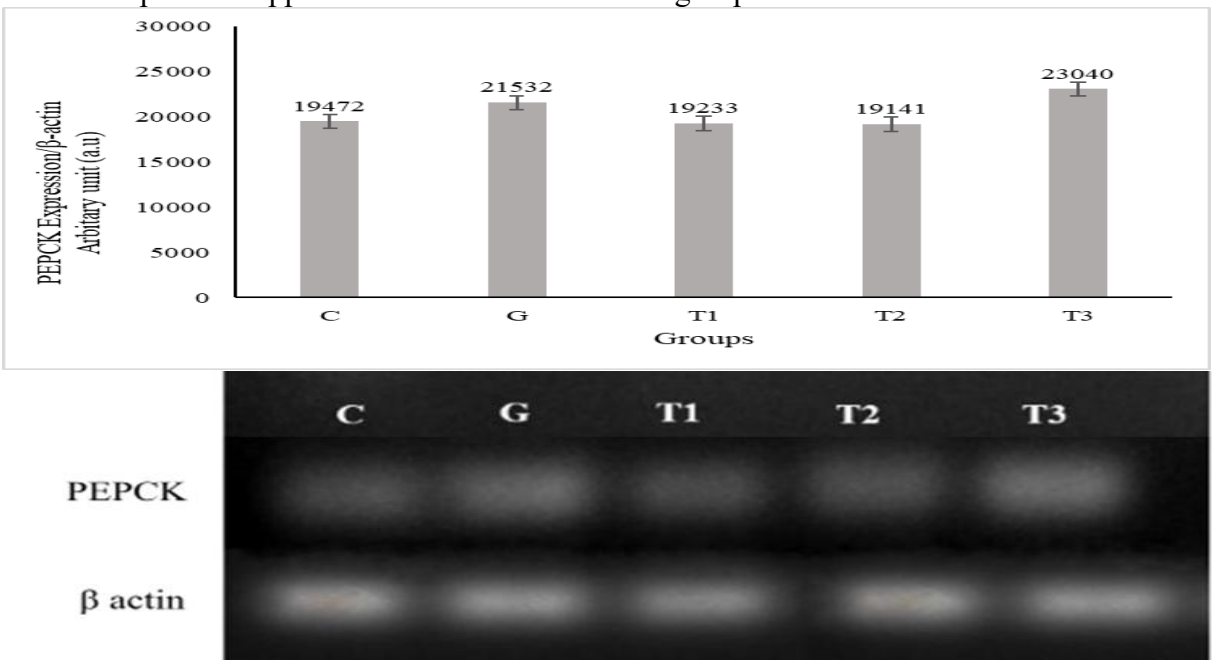
The body length of five zebrafish larvae aged 72-144 hpf was observed and randomly selected for each group (Khotimah et al., 2022). Body length observations of the zebrafish larvae were captured using an Olympus SZ61 stereo microscope linked to an Optilab camera

2.2.1. Body length measurements of zebrafish larvae were performed using images analyzed using calibrated ImageJ software. The body length of the zebrafish larvae was measured in millimeters (mm), starting at the tip of the nose, and ending at the caudal fin. Apoptosis in the ocular tissue of zebrafish larvae was visualized using fluorescence microscopy and quantified using ImageJ software. Ocular apoptosis was assessed in five zebrafish larvae at 96 hpf, that were randomly selected from each experimental group.

The blood flow of zebrafish larvae was observed in five larvae aged 72–96 hpf, which were randomly selected for each group. The blood flow of larval zebrafish was observed using a stereo microscope (Olympus CX21) with 100x total magnification and connected to a recording device (Panasonic Lumix GF8 camera). The video recording of the zebrafish larvae's blood flow was analyzed using Danioscope software. The heart rate of the zebrafish larvae was observed using a stereo microscope (Olympus CX21) with 100x total magnification and connected to a recording device (Panasonic Lumix GF8 camera). The heart rate of zebrafish larvae was observed in five larvae aged 72–144 hpf, which were randomly selected for each group. The heart rate was calculated for 1 min using the Danioscope software.

3. RESULTS AND DISCUSSION

The results of this study indicate that Curcuma longa extract nanoemulsion preparation can improves the developmental process in high glucose-induced zebrafish (Danio rerio) embryos. the novelty in this study lies in the treatment preparation made into nanoemulsion so that with the smallest concentration at a concentration of 1.25 µg/mL can decrease PEPCK expression and prevent developmental process disorders. Phosphoenolpyruvate carboxykinase (PEPCK) expression was used to confirm the hyperglycemic state of the zebrafish larvae. The results of PEPCK expression measurement in zebrafish larvae at 72 hpf in the control, glucose, and treatment groups at doses of 1.25 µg/ml, 2.5, and 5 µg/ml of Curcuma longa extract are presented in Figure 1. PEPCK expression in zebrafish larvae at 72 hpf increased following exposure to 3% glucose solution. A nanoemulsion of Curcuma longa extract resulted in decreased PEPCK expression compared to the glucose group, with the most pronounced effect observed in the T1 group (1.25 µg/mL nanoemulsion of Curcuma longa extract), wherein PEPCK expression approximated that of the control group.



Description: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract.*

Figure 1. PEPCK expression of zebrafish larvae at 72 hpf.

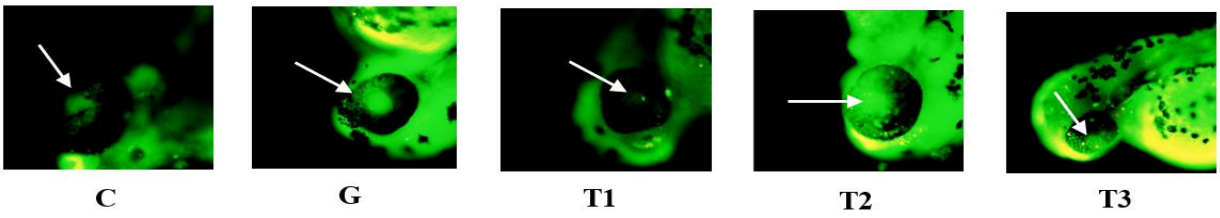
The mean body length of zebrafish larvae aged 72 hpf to 144 hpf in the control, glucose, and treatment groups at doses of 1.25 µg/ml, 2.5, and 5 µg/ml nanoemulsion of *Curcuma longa* extract are presented in Table 1 and Figure 2. The body length of zebrafish larvae at 72–144 hpf exhibited a statistically significant decrease following exposure to 3% glucose solution. A nanoemulsion of *Curcuma longa* extract induced a statistically significant increase in body length compared to the glucose group, notably in the T1 group (1.25 µg/mL nanoemulsion of *Curcuma longa* extract), which demonstrated a statistically comparable increase in body length to that observed in the control group ($p < 0.05$).

Table 1. The Average of the Body Length of Zebrafish Larvae Aged 72 hpf to 144 hpf.

Time	C	G	T1	T2	T3
72 hpf	3.40 ± 0.09*	3.13 ± 0.05	3.31 ± 0.12*	3.23 ± 0.04*	3.26 ± 0.10*
96 hpf	3.54 ± 0.11*	3.16 ± 0.10	3.46 ± 0.14*	3.49 ± 0.10*	3.34 ± 0.16*
120 hpf	3.85 ± 0.37*	3.19 ± 0.37	3.50 ± 0.14*	3.41 ± 0.30*	3.50 ± 0.34*
144 hpf	3.96 ± 0.08*	3.44 ± 0.24	3.92 ± 0.23*	3.59 ± 0.08*	3.54 ± 0.51*

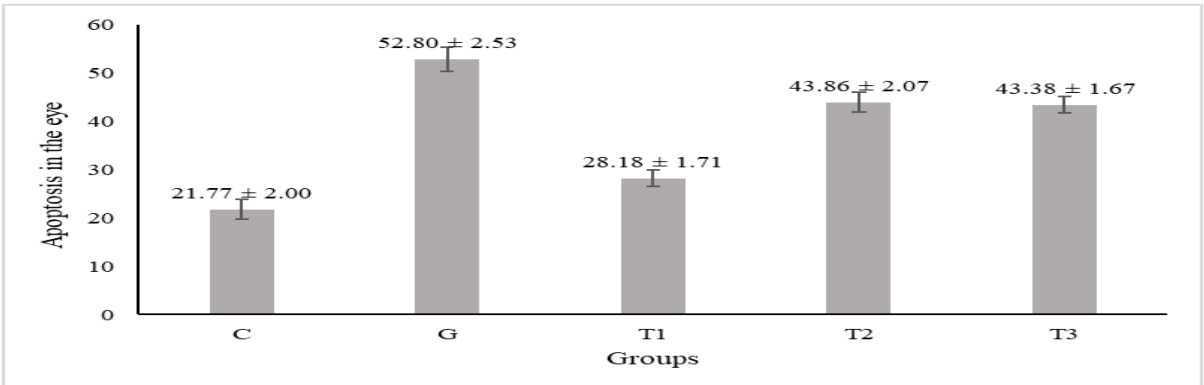
Information: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract. *: p<0.05 compared with the 3% glucose group.*

The results of the mean apoptosis measurements in the eyes of zebrafish larvae at 96 hpf in the control, glucose, and treatment groups at doses of 1.25 µg/ml, 2.5, and 5 µg/ml nanoemulsion of *Curcuma longa* extract are presented in Figures 3 and 4. Apoptosis in the eyes of zebrafish larvae at 96 hpf was significantly increased following exposure to 3% glucose solution. A nanoemulsion of *Curcuma longa* extract significantly reduced eye apoptosis compared to the glucose group, notably in the T1 group (1.25 µg/mL nanoemulsion of *Curcuma longa* extract), which demonstrated a decrease in eye apoptosis to a level statistically comparable to that of the control group ($p < 0.05$).



Information: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract.*

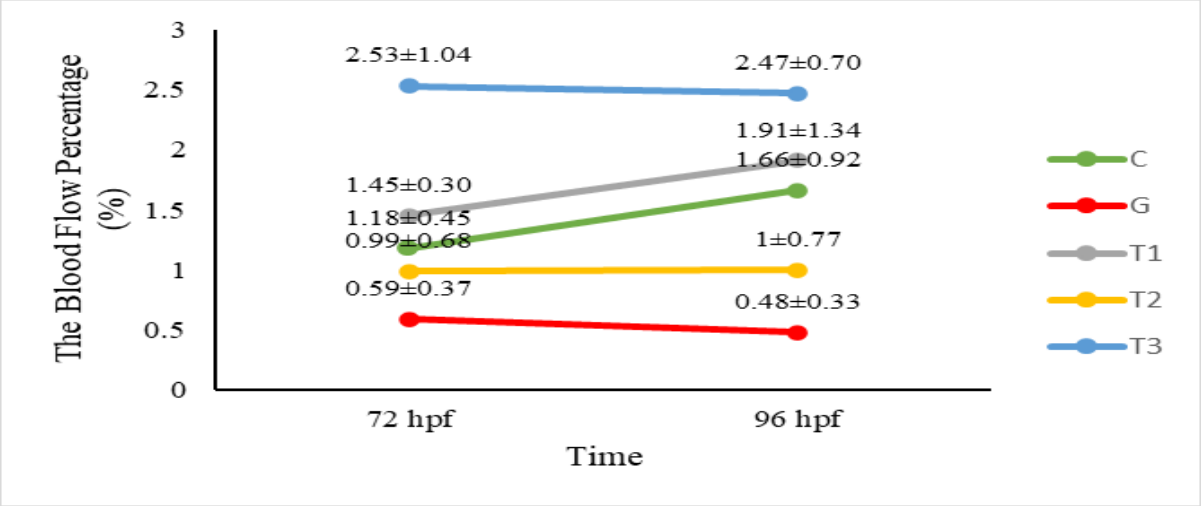
Figure 2. The apoptosis in the eye at 96 hpf zebrafish larvae using a fluorescence microscope.



Information: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract.*

Figure 3. The average of apoptosis in the eye at 96 hpf zebrafish larvae.

The mean blood flow percentage of zebrafish larvae aged 72-96 hpf in the control, glucose, and treatment groups at doses of 1.25 µg/ml, 2.5, and 5 µg/ml nanoemulsion of *Curcuma longa* extract are presented in Figure 5. The blood flow of zebrafish larvae at 72–96 hpf exhibited a significant decrease following exposure to 3% glucose solution. A nanoemulsion of *Curcuma longa* extract demonstrated a significant increase in blood flow compared to the glucose group, notably in the T1 group (1.25 µg/mL nanoemulsion of *Curcuma longa* extract), which elevated blood flow to a level significantly similar to that of the control group ($p < 0.05$).



Information: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract.*

Figure 4. The average of blood flow percentage at 72 hpf and 96 hpf zebrafish larvae.

The average heart rate of zebrafish larvae aged 72–144 hpf in the control, glucose, and treatment groups at doses of 1.25 µg/ml, 2.5, and 5 µg/ml nanoemulsion of *Curcuma longa* extract are presented in Table 2 and Figure 6. The heart rate of zebrafish larvae at 72–144 hpf exhibited a significant decrease following exposure to 3% glucose solution. A nanoemulsion of *Curcuma longa* extract, particularly in the T1 group (1.25 µg/mL nanoemulsion of *Curcuma longa* extract), demonstrated a significant increase in heart rate, approximating the value observed in the control group ($p < 0.05$).

Table 2. The Average of The Heart Rate of Zebrafish Larvae Aged 72 hpf to 144 hpf

Time	C	G	T1	T2	T3
72 hpf	136.80 ± 8.98*	110.80 ± 10.84	203.00 ± 10.70*	200.40 ± 3.36*	147.80 ± 10.66*
96 hpf	190.20 ± 7.75*	153.60 ± 5.36	202.80 ± 15.48*	155.60 ± 8.01*	127.60 ± 11.23*
144 hpf	271.00 ± 10.17*	205.80 ± 9.14	267.40 ± 10.66*	233.80 ± 4.32*	204.40 ± 35.43*

Information: *C: Control, G: 3% glucose, T1: 3% glucose and 1.25 µg/mL nanoemulsion of Curcuma longa extract, T2: 3% glucose and 2.5 µg/mL nanoemulsion of Curcuma longa extract, and T3: 3% glucose and 5 µg/mL nanoemulsion of Curcuma longa extract. *: p<0.05 compared with the 3% glucose group.*

High glucose levels, particularly in gestational diabetes, can lead to developmental complications in embryos. Such complications can include growth retardation, heart and neural defects, and other malformations. These disruptions occur due to elevated oxidative stress and inflammation, both of which are associated with hyperglycemia. Gestational diabetes mellitus (GDM) develops in the second and third trimesters due to reduced insulin sensitivity caused by hormones, such as hPL, estrogen, and progesterone (Berberoglu, 2019; Choudhury &

Rajeswari, 2021). GDM patients experience elevated oxidative stress, and glucose intolerance adversely affects the fetus (Phoswa & Khaliq, 2021; Prakash et al., 2017). Hyperglycemia increases PEPCK expression, facilitating gluconeogenesis (Brearley et al., 2020; Runtuwene et al., 2016). GDM-induced glucagon activates PGC1α and enhance gluconeogenic genes. Hyperglycemia-induced ROS trigger oxidative stress pathways, causing insulin resistance and increasing PEPCK and G6Pase levels, thereby elevating hepatic glucose (Kang et al., 2020; Xiong et al., 2016).

Zebrafish larvae exposed to 3% glucose have shorter body lengths due to hyperglycemia-induced ROS, which causes oxidative stress, reduces ATP synthesis, and disrupts bone growth (Millward, 2017; Wijayanti et al., 2021). Hyperglycemia also leads to pancreatic beta-cell damage, insufficient insulin secretion, insulin resistance, and growth inhibition (Rolfe et al., 2018). Hyperglycemia induces apoptosis, which significantly affects the eyes (Zhang et al., 2013). Progressive apoptosis disrupts the blood-retinal barrier, causing macular edema and visual disturbances (Kowluru & Mishra, 2015; Mugoni et al., 2014). Elevated glucose levels alter apoptotic protein levels, activate caspases, and increase proteolytic activity, culminating in eye apoptosis (Kumar et al., 2015). Exposure to 3% glucose reduced blood flow in zebrafish larvae due to hyperglycemia-induced ROS elevation, affecting endothelial function and vessel dilation. The larvae also exhibited a decrease in heart rate, which was attributed to hyperglycemia-induced oxidative stress (Fiorentino et al., 2013). Chronic hyperglycemia causes vascular abnormalities, activates p-53, induces apoptosis, hypertrophy, and atrial remodeling, resulting in pathological fetal heart rates (Al-Biltagi et al., 2021; Raut et al., 2016).

In this context, natural compounds like curcumin, which have antioxidant and anti-inflammatory properties, present a promising therapeutic option. However, curcumin is known to have low bioavailability, meaning it is poorly absorbed when taken orally. To address this limitation, the researchers used nanoemulsion technology to improve curcumin's delivery to target tissues. Nanoemulsions are tiny droplets of oil-based curcumin suspended in water, which allows for better absorption and distribution in the body. This approach helps harness the therapeutic potential of curcumin more effectively. Nanoemulsions can increase the antidiabetic effect of okra extract through increasing active compounds into the interstitial space so that absorption increases. Besides, turmeric (*Curcuma longa*) is a natural antioxidant with curcumin as its main active compound. Curcumin has been shown to help in reducing oxidative stress and inflammation, both of which are key contributors to developmental defects induced by high glucose. By enhancing the bioavailability of curcumin using a nanoemulsion, the study aims to explore its therapeutic effects on improving the development of zebrafish embryos exposed to high glucose levels. Studies have shown that *Curcuma longa* extract has anti-inflammatory and antidiabetic effects (Chuengsamarn et al., 2012). Nanoemulsions improve the bioavailability and absorption of curcumin. This study found that a nanoemulsion of the *Curcuma longa* extract (1.25 µg/mL) reduced PEPCK expression. The antidiabetic mechanism of curcumin includes promoting adipocyte differentiation, activating PPAR-γ, reducing liver glucose production via AMP kinase activation, and PEPCK inhibition (Sovia et al., 2011). Additionally, curcumin increases hepatic glycogen levels by inhibiting GSK-3β and suppresses gluconeogenesis by inhibiting G6Pase and PEPCK (Shen et al., 2017).

A *Curcuma longa* extract nanoemulsion (1.25 µg/mL) increased body length. Curcumin inhibits ROS elevation, protects β-pancreatic cells, and maintains IGF-1 and GH levels, which are critical for growth (Larasati et al., 2018; Marton et al., 2021). However, nanoemulsion concentrations of 2.5 and 5 µg/mL caused curved tails and bodies in zebrafish due to curcumin toxicity. Proper concentration management is essential, as the nanoemulsion mitigates developmental defects such as curled tails, curved bodies, pericardial edema, shorter body length, and mortality in zebrafish (Sachett et al., 2022). The nanoemulsion (1.25 µg/mL) also

reduced apoptosis in the ocular tissue by neutralizing free radicals and preventing ROS-induced apoptosis. Elevated antioxidant enzyme levels correlate with increased Bcl-2 expression, mitigating the oxidative stress characteristic of diabetes. Curcumin preserves Bcl-2's antioxidant and antiapoptotic properties (El-Bahr, 2013). Furthermore, the nanoemulsion (1.25 µg/mL) enhanced blood flow by scavenging ROS, maintaining endothelial function, and preserving nitric oxide (NO) production (Łuczak et al., 2020). It also increases the heart rate as curcumin inhibits ROS, enhancing NO, a positive chronotropic molecule. Higher concentrations (2.5 and 5 µg/mL) reduced the zebrafish heart rate, indicating potential toxicity.

Nanoemulsions improve bioavailability by stabilizing and enhancing the absorption of bioactive herbal compounds, thus requiring careful concentration management to avoid toxicity (Harwansh et al., 2019; Jaiswal et al., 2015). This study demonstrates that the nanoemulsion of *Curcuma longa* extract, particularly its active compound curcumin, can effectively improve the developmental process of zebrafish embryos exposed to high glucose. The nanoemulsion delivery method enhances the bioavailability of curcumin, allowing it to exert its therapeutic effects more efficiently. The improvement in developmental outcomes, the reduction of oxidative stress, and the alleviation of morphological abnormalities highlight curcumin's potential as a therapeutic agent for managing developmental issues caused by high glucose exposure, such as those seen in gestational diabetes. The findings suggest that curcumin, when delivered via nanoemulsion, holds promise as a potential intervention for mitigating the developmental effects of diabetes and other conditions associated with high glucose. This study only examined the process of fetal development due to GDM. Recommendation for future research is needed to explore the full therapeutic potential of curcumin in human applications, particularly in the context of maternal diabetes and its impact on fetal development. Based on these findings, the author intends to investigate the precise therapeutic window of the nanoemulsion and its molecular effects on embryonic development in future studies.

4. CONCLUSION

Our data suggest that a nanoemulsion of Curcuma longa extract at a concentration of 1.25 µg/mL can decrease PEPCK expression and prevent developmental process disorders by increasing body length, decreasing apoptosis in the eye, and maintaining blood flow, and heart rate in high glucose-induced zebrafish (Danio rerio) embryos. These findings open avenues for further exploration into the nanoformulation of herbal bioactives as potential therapeutic agents for hyperglycemia-induced developmental complications. Future research should focus on elucidating the underlying molecular mechanisms and evaluating the pharmacokinetics and bioavailability of the nanoemulsion in more complex in vivo systems, including mammalian models. Furthermore, the scalability of nanoemulsion formulation and its safety profile warrant comprehensive investigation prior to potential clinical translation. Ultimately, this study provides a foundational step toward the development of novel, plant-based interventions for managing gestational diabetes and related metabolic disorders, with potential applications in maternal-fetal medicine and pediatric endocrinology.

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