Biomedical Journal of Indonesia
Journal Homepage: https://bji-fk.ejournal.unsri.ac.id

Effect of Curcumin Nanoemulsion on Brain Cell Development, Locomotor Function, and Mortality of Zebrafish Larvae Model of Gestational Diabetes Mellitus

Subandi[1*], Mutiara Syifa Azahra[2], Amarsya Ovinery[3], Qorry Afyu Akhmalia[4], Aliyah Tresna Asih[5], Hikmawan Wahyu Sulistomo[6], Nurdiana[7], I Wayan Arsana Wiyasa[8]

[1,8]Department of Midwifery, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[2,3,4,5]Midwifery Undergraduate Study Program, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[6,7]Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, Indonesia

ARTICLE INFO

Keywords:
Curcumin nanoemulsion
Gestational diabetes
Zebrafish

Corresponding author:
Subandi

E-mail address:
desobg@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/BJI.v11i3.270

ABSTRACT

Introduction. Gestational diabetes mellitus (GDM) is glucose intolerance in pregnancy due to reduced ability of pancreatic beta cells to produce insulin, causing oxidative stress that triggers various complications such as brain apoptosis to locomotor disorders and decreased head size which has an impact on mortality rates. Pharmacological treatment of GDM has side effects that risk affecting fetal development. Therefore, curcumin as a herbal medicine can be an option for GDM treatment because it can increase insulin sensitivity by activating the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) receptor. **Methods.** Post Test Only Control Group Design was used in this experimental study which was tested on each research variable consisting of 25 zebrafish embryos divided into 5 treatment groups consisting of a negative control, a positive control exposed to 3% glucose, and a diabetic group given curcumin nanoemulsion with three different doses (0.3125 µg/ml, 0.625 µg/ml, 1.25 µg/ml). One Way ANOVA and Post Hoc Tukey tests were used to analyze the data from this study. **Results.** P value from One Way ANOVA test p=0.024 for brain apoptosis, p=0.00 for locomotor ability, p=0.04 for head size, and p=0.006 for mortality rate. Turkey Post Hoc test showed significant differences in the control and 3% glucose + 0.625 µg/ml curcumin nanoemulsion groups (p<0.05). **Conclusion.** Curcumin nanoemulsion therapy has an effect on decreasing brain apoptosis, increasing locomotor, inhibiting the decrease in head size, and decreasing the mortality rate in zebrafish with gestational diabetes. The most effective dose of curcumin nanoemulsion is 0.625 µg/ml.

1. Introduction

Gestational diabetes mellitus (GDM) is a glucose intolerance disorder caused by a decrease in the ability of the beta pancreas to secrete insulin and is found in pregnant women who have never been diagnosed with diabetes and show high glucose levels during pregnancy.[1] According to the International Diabetes Federation (IDF) in 2021, hyperglycemia in pregnancy affects 16.7% of all pregnancies worldwide, with 80.3% of them caused by gestational diabetes mellitus with the highest incidence in Asia and the Pacific Islands at 24.2%.[2,3] The prevalence of DMG in Indonesia according to WHO of 1.9% -3.6%. Meanwhile, of all pregnancies that occur 1-14% experience DMG and those that are not diagnosed range from 10-25%. According to estimates, 3-5% of 135,000 pregnant women experience DMG each year. If not managed properly, this condition can increase the risk of perinatal complications and metabolic diseases in mothers and children.[1]

GDM is usually diagnosed when entering the second trimester of pregnancy or more than 20 weeks of pregnancy, where at that time there is an increase in the hormones estrogen, progesterone, and human placental lactogen (HPL) which have the opposite effect to insulin.[4] Uncontrolled increase in insulin resistance causes hyperglycemia during pregnancy which can cause oxidative stress that triggers cell apoptosis. High glucose levels can increase Reactive Oxygen Species (ROS) which can make cell and tissue antioxidants and the oxidative system unbalanced and can cause disruption of glucose homeostasis. This condition can trigger disruption of glucose transport metabolism through facilitated diffusion in the placenta and fetal brain, causing malformations in the brain during the organogenesis process.[5]

Hyperglycemia triggers oxidative stress, thereby reducing antioxidant enzymes.[6] Mothers who experience hyperglycemia can be passed on to the fetus through placental transfer. Oxidative stress can damage cellular mechanisms and deoxyribonucleic acid (DNA) structures that induce cell apoptosis.[7] Hyperglycemia in the fetus due to DMG can cause growth disorders, apoptosis, congenital malformations, and fetal death.[8] Exposure to high glucose can also reduce dopaminergic neuron activity and increase brain cell apoptosis, thereby contributing to decreased locomotory function.[9] In addition, neurons that control muscles experience impaired signal transmission from nerves to muscles due to apoptosis, causing uncoordinated or weak movements, which are called hypotonia (floppy infant syndrome).[10]

The main treatment for DMG patients can be done with insulin therapy. In addition to insulin, it can also be done with drugs such as metformin and glyburide. However, the safety of these two drugs is still not available because these drugs cross the placenta.[11,12] Therefore, other safer therapies are needed to prevent adverse effects on the fetus or long-term side effects due to continuous drug consumption.[13] One of these other treatments is with herbal medicines such as curcumin or turmeric. Turmeric has antioxidant compounds in the form of natural flavonoids contained in curcumin. These flavonoids have various pharmacological functions including antidiabetic, anti-inflammatory, antioxidant, antiangiogenic, anticarcinogenic, antiapoptogenic, antimicrobial, and neuroprotective.[14] Antidiabetic in curcumin can lower glucose levels and increase insulin sensitivity by repairing pancreatic beta cells.[15]

However, it is known that curcumin has properties that are difficult to dissolve in water and have poor bioavailability in metabolic absorption.[16] Therefore, a revolution in the size of curcumin is needed to increase absorption in the body with micro or nano techniques. One of them is like a nanoemulsion that is finely dispersed in water or oil.[17,18]

Diabetes research, an experimental animal model is needed to help determine the pathogenesis of the disease and drug development. One of the experimental animal models that is suitable for diabetes research studies is zebrafish. Zebrafish are vertebrates that have characteristics of endocrine gland function and cell metabolism similar to humans. In addition, there are other advantages of this fish, such as rapid growth, simple fish cultivation, embryos that develop outside their mothers, and transparent development processes.[19] This can facilitate observation in determining the development of fish to assess the toxicity of teratogenicity of drug development. Thus, this fish model is very suitable for research on the effect of curcumin in diabetes.[20]

Previous research by Ilham, 2018, it was stated that giving 5% glucose to zebrafish embryos can reduce the size of the larval head and increase the mortality of zebrafish larvae.[21] In addition, from the research of Fathir, 2022, it was stated that there was a decrease in body length in embryos exposed to 3% glucose. And the results of the study stated that giving nanocurcumin at a dose of 1.25 µg/ml was effective in preventing a decrease in the body length of zebrafish larvae due to glucose exposure.[22]

Based on the description above, it is possible that giving curcumin nanoemulsion has an effect on lowering blood glucose which can reduce brain apoptosis so that it can prevent congenital malformations, motor disabilities, and death due to gestational diabetes mellitus. Therefore, this study aims to evaluate the effect of giving curcumin nanoemulsion on brain cell development, movement, and mortality of zebrafish larvae (*Danio rerio*) with a gestational diabetes mellitus model.

2. Methods

This research used an experimental study with post test only control group design which was tested on each research variable consisting of 25 zebrafish embryos divided into 5 treatment groups. Zebrafish embryos aged 2 hours post fertilization (hpf) were induced with 3% glucose then given curcumin nanoemulsion doses of 0.3125 µg/ml; 0.625 µg/ml; 1.25µg/ml. The doses were determined because in a previous study conducted by Iskandar (2022), doses of 2.5 µg/ml and 5 µg/ml were not optimal in providing therapeutic effects, causing toxic effects in test animals. A glucose concentration of 3% was chosen by the researchers because previous studies have shown that exposure to 3% glucose for 48 hours can produce zebrafish embryos with a model of gestational diabetes mellitus[26,27].

Brain Apoptosis

Brain cell apoptosis was observed at 72 hpf using acridine orange staining and fluorescence microscopy. Head measurements were taken at 72 hpf uses of an olympus microscope and measured through Image Raster 3 software. The light reflection, which appears as small green dots around the larva's head, is measured using freehand selection tools. After that, the average area can be seen in the analyze menu in the measure section. The number that appears shows the average area of apoptosis in the larva's brain in terms of cell density.

Motility and Locomotor

Measurement of zebrafish larval motility was carried out by touching the tail of the larva using the tip of a needle, then observing the larva's movement response to move away from the stimulus whether it was motile (+) or not motile (-). Locomotor measurements included the level of movement and activity of the larvae to move. After being stimulated at the tip of the tail using a needle, the movement of the larvae was observed for 1 minute on a 4x6 well

plate, each of which had been divided into 4 equal parts, then the number of lines that the larvae could pass through was calculated. After that, a comparison of motility and locomotory between the control group and the group of larvae that were treated was carried out.

Head Size

Observations of head size were carried out when the larvae were 72 hpf with the aid of an Olympus microscope and measured using Image Raster 3 software by placing the larvae in a dorsal position and measuring the vertical diameter of the larva's head.

Mortality Rate

Mortality rate measurements were observed at 48, 72, and 96 hpf with the mortality rate formula. The motility of zebrafish larvae was measured by touching the tail of the larva with the tip of a needle, then observing the larva's response to move away from the stimulus to determine whether it was motile (+) or immotile (-).

The four studies have been registered in the research ethics number of the Faculty of Medicine, Brawijaya University with number 347/EC/KEPK-S1-KB/10/2024 for motility and locomotor experience, 368/EC/KEPK-S1-KB/10/2024 for head size experience, 361/EC/KEPK-S1-KB/10 /2024 for mortality rate experience, and 360/EC/ KEPK-S1-KB/10/2024 for apoptosis brain experience.

3. Results

Effects on Motility and Locomotor

Zebrafish larva motility was measured by touching the larva's tail with a needle tip, then observing the larva's movement response to avoid the stimulus, whether motile (+) or non-motile (-). Locomotor measurement includes the level of movement and activity of the larva to change location. After stimulating the tip of the tail with a needle, the larva's movement was observed for 1 minute on a 4x6 plate, where each well was divided into four equal parts by lines, and the number of lines the larva could traverse was counted. Subsequently, comparisons of motility and locomotor activity were made between the control group and the treated larva group.

The results of motility observations showed that all samples in each treatment group exhibited positive motility (100%), indicating no differences in motility between groups (Table 1). The observed differences were in the type of larval movement after stimulation. Group K- exhibited strong movement, while group K+ exhibited weak movement. Groups P1 and P2 showed strong movement, with P2 almost resembling the K-response. Meanwhile, group P3 showed a weak movement response. Weak movement is defined as the larvae's response to stimulation by moving away from the stimulation point without the ability to swim actively. This also influenced the results of the larvae's locomotor calculations (Table 2).

The P3 group data were not normally distributed ($p<0.05$), so the analysis used the Kruskal-Wallis test, which showed significant results ($p=0.000$), meaning that there were differences in the means between groups. This indicates that the administration of curcumin nanoemulsion affects the locomotor activity of zebrafish larvae with a gestational diabetes model. The Pairwise Comparison test showed that K+ was significantly different from K- ($p=0.002$), indicating a decrease in locomotor activity due to 3% glucose. There were no significant differences between K- and P1 ($p=0.008$) or K- and P2 ($p=0.968$), meaning that doses of 0.3125 and 0.625 µg/ml curcumin were able to restore locomotor function to near-normal levels. However, P3 was significantly different from K- ($p=0.000$), indicating that the 1.25 µg/ml dose actually reduced locomotor ability. There was a significant difference between K+ and P2 ($p=0.002$), indicating that the 0.625 µg/ml dose effectively improved locomotor function. Conversely, the 1.25 µg/ml dose (P3) did not differ significantly from K+ and showed lower values, indicating potential toxicity despite not being statistically significant. The low insulin levels in P3 support the hypothesis of high-dose toxicity.

In contrast, Khotimah et al. (2022), stated that the 1.25 µg/ml dose is optimal for diabetes. This difference may be due to differences in the parameters assessed, namely cardiac function vs. locomotor function, which are influenced by different mechanisms. High doses may be cardioprotective but neurotoxic. Curcumin acts as an antioxidant by inhibiting pro-oxidant enzymes and increasing endogenous antioxidant enzymes such as SOD, catalase, and glutathione peroxidase. This protects motor neurons and pancreatic cells from oxidative stress, maintains insulin production, and enhances glucose and ATP metabolism, ultimately improving the locomotor ability of larvae.

Table 1. Effect on motility rate

Group	Motility (+/-)	Percentage
K-	+	100%
K+	+	100%
P1	+	100%
P2	+	100%
P3	+	100%

Note : K- = control group; K+ = 3% glucose group; P1 = 3% glucose group + curcumin nanoemulsion at a dose of 0.3125 µg/ml; P2 = 3% glucose group + curcumin nanoemulsion at a dose of 0.625 µg/ml; P3 = 3% glucose group + curcumin nanoemulsion at a dose of 1.25 µg/ml

Table 2. Effect on locomotion

Group	Mean ± SD
K-	5,857 ± 2,410[a]
K+	1,000 ± 0,816[b]
P1	1,428 ± 0,975[ab]
P2	5,428 ± 1,618[a]
P3	0,285 ± 0,487[b]

Note: Letter notation indicates significance between groups; K- = control group; K+ = 3% glucose group; P1 = 3% glucose group + curcumin nanoemulsion at a dose of 0.3125 µg/ml; P2 = 3% glucose group + curcumin nanoemulsion at a dose of 0.625 µg/ml; P3 = 3% glucose group + curcumin nanoemulsion at a dose of 1.25 µg/ml

Effect on Mortality Rate

In this study, exposure was administered to 24 hpf zebrafish embryos. A total of 25 embryos were placed in a 48-well plate, with one embryo in each well. The treatment groups included a negative control, 3% glucose, and 3% glucose with the addition of curcumin nanoemulsion at doses of 0.3125 µg/ml, 0.625 µg/ml, and 1.25 µg/ml. Exposure was maintained until 96 hpf, with mortality rates observed at 48, 72, and 96 hpf.

Results showed that exposure to 3% glucose significantly increased mortality at all three observation times ($p < 0.05$) compared to the negative control. At 48 hpf, mortality increased in the groups with added curcumin at doses of 0.3125 µg/ml and 1.25 µg/ml. However, at 72 and 96 hpf, both doses significantly reduced mortality ($p < 0.05$). The 0.625 µg/ml dose was not significantly different from the negative control but was significantly different from the positive control.

At 96 hpf, all doses of curcumin nanoemulsion significantly reduced mortality compared to the 3% glucose group alone, indicating the potential of curcumin to mitigate the effects of glucose toxicity.

Statistical analysis showed that the data at 72 and 96 hpf met the criteria for one-way ANOVA, with significant results ($p = 0.012$ and $p = 0.006$). The Tukey HSD post hoc test showed significant differences between the positive and negative controls ($p = 0.012$ and $p = 0.005$), as well as between the positive control and the 0.625 µg/ml dose ($p = 0.034$). However, at doses of 0.3125 µg/ml and 1.25 µg/ml, the differences with the positive control were not significant. The highest mortality was found in the positive control group, while the 1.25 µg/ml dose tended to show higher mortality rates than lower doses.

At 48 hpf, since the data did not meet the assumptions of normality and homogeneity, the Kruskal-Wallis test ($p = 0.049$) and Mann-Whitney test were used. The results showed that mortality in the glucose group and the two curcumin doses (0.3125 and 1.25 µg/ml) differed significantly from the control. However, no significant differences were found between the curcumin groups, indicating inconsistent effects at the early stage of observation.

Effect on Head Size

Based on the results of the study, the administration of 3% glucose to zebrafish embryos had an effect on the reduction in the size of the larvae's heads. The average head size in the control group (K-) was 0.490, while in the group exposed to 3% glucose, it decreased to 0.384. Statistical analysis revealed a significant difference between the two groups ($p = 0.003$; $p < 0.05$). These findings are consistent with the research by Ilham (2018), which showed a reduction in larval head size due to exposure to 5% glucose, and Fathir (2022), who reported a reduction in larval body length at 3% glucose.

The results of the normality test using Shapiro-Wilk showed that the head size data were normally distributed ($p = 0.118$), and the results of Levene's homogeneity test showed homogeneous data distribution ($p = 0.259$). Since the assumptions were met, a One-Way ANOVA test was conducted, which showed a significant difference between groups ($p = 0.004$), indicating the effect of curcumin nanoemulsion administration on the head size of zebrafish larvae in the gestational diabetes mellitus model.

Table 3. Average effect of curcumin nanoemulsion exposure on zebrafish embryo mortality rate

Exposure	Mortality Rate (hpf)		
	48	72	96
K (+)	0%[a]	5%[a]	8%[a]
K (-)	14%[b]	39%[b]	91%[b]
P1	19%[a]	24%[a]	54%[a]
P2	10%[a]	11%[b]	35%[a]
P3	19%[a]	29%[a]	75%[b]

Note: Letter notation indicates significance between groups; K- = control group; K+ = 3% glucose group; P1 = 3% glucose group + curcumin nanoemulsion at a dose of 0.3125 µg/ml; P2 = 3% glucose group + curcumin nanoemulsion at a dose of 0.625 µg/ml; P3 = 3% glucose group + curcumin nanoemulsion at a dose of 1.25 µg/ml. Different letters in the notation indicate significant differences ($p < 0.05$). The same letters indicate no significant differences ($p > 0.05$). hpf (hours post fertilization): the number of hours after fertilization occurred

Table 4. The Effect of glucose on larvae head size

Group	Larvae Head Size, Mean ± SD
K-	0,490 mm ± 0,049 [a]
K+	0,384 mm ± 0,029 [b]
P1	0,439 mm ± 0,033 [ab]
P2	0,479 mm ± 0,060 [a]
P3	0,444 mm ± 0,067 [ab]

Note: Letter notation indicates significance between groups; K- = control group; K+ = 3% glucose group; P1 = 3% glucose group + curcumin nanoemulsion at a dose of 0.3125 µg/ml; P2 = 3% glucose group + curcumin nanoemulsion at a dose of 0.625 µg/ml; P3 = 3% glucose group + curcumin nanoemulsion at a dose of 1.25 µg/ml

The Post Hoc Tukey test showed a significant difference between the control group and the 3% glucose group ($p = 0.003$), but no significant difference between the control group and the P1, P2, and P3 treatment groups. The 3% glucose group had a significant difference with the P2 group ($p = 0.01$), but no significant difference with P1 and P3.

Overall, administration of curcumin nanoemulsion at all three doses was able to inhibit the reduction in head size caused by glucose exposure, with the 0.625 µg/ml dose showing the highest efficacy. Doses that are too low or too high tend to be less effective and may be toxic to embryonic development.

Effect on apoptosis in the brain

Zebrafish embryos aged 72 hpf were stained with acridine orange (Sigma-Aldrich) 5 µg/ml in the embryo medium for 1 hour under dark conditions. After that, the zebrafish embryos were rinsed with embryo medium five times and anesthetized before visualization. The fluorescence intensity of each zebrafish embryo will be quantified using the Image J program. Apoptosis in the brain will be indicated by the presence of light emission in the head region, suggesting neurotoxicity in the brain.

After the samples were observed using a fluorescence microscope and photographed, the photos were analyzed using Image J and SPSS 30.0 software, with a total of 25 photos. This study involved 5 treatment groups, each consisting of 5 zebrafish embryos, with treatment administered from the embryonic stage and apoptosis analysis conducted at the 72 hpf larval stage .

The observation results showed an increase in green fluorescence in the brain of the positive control group (3% glucose) compared to the negative control group (Figure 2). A decrease in fluorescence intensity approaching the negative control was observed in the glucose + nanoemulsion curcumin 0.3125 µg/ml and 0.625 µg/ml groups. Meanwhile, in the 1.25 µg/ml dose group, the fluorescence intensity appeared higher than the two previous doses.

The Shapiro-Wilk normality test indicated that the data were normally distributed. The homogeneity test was also met. One-way ANOVA analysis showed a significance value of 0.024 ($p < 0.05$), indicating a significant difference between groups. Post hoc tests showed that only groups 1 and 2 had significant differences ($p < 0.05$).

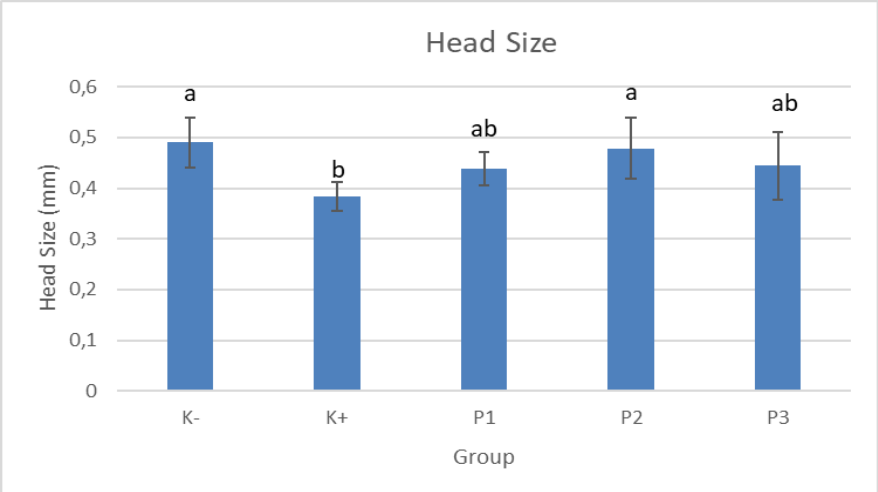

Figure 1. The Effect of curcumin nanoemulsion administration on larvae size

K- = control group; K+ = 3% glucose group; P1 = 3% glucose group + curcumin nanoemulsion at a dose of 0.3125 µg/ml; P2 = 3% glucose group + curcumin nanoemulsion at a dose of 0.625 µg/ml; P3 = 3% glucose group + curcumin nanoemulsion at a dose of 1.25 µg/ml

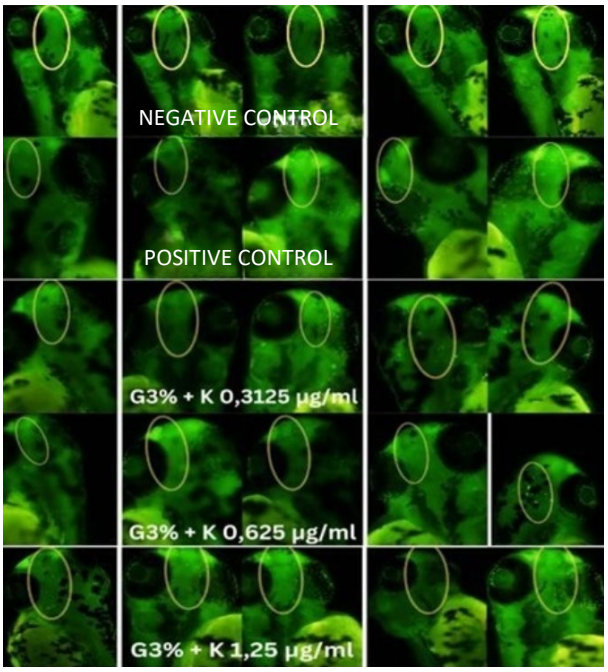

Figure 2. Results of observations on the brains of zebrafish larvae using a fluorescence microscope

4. Discussion

This study aimed to evaluate the impact of 3% glucose exposure on the development of zebrafish embryos and larvae as a model of gestational diabetes mellitus (GDM), and to explore the potential protection of curcumin nanoemulsions at various doses. Glucose exposure was shown to significantly increase the mortality rate of zebrafish embryos at 48, 72, and 96 hours post fertilization (hpf) compared to the control group. This increase in mortality is thought to be caused by oxidative stress resulting from the accumulation of Reactive Oxygen Species (ROS), which disrupts cellular structures and triggers the apoptosis pathway through the activation of the Bax protein and a decrease in the Bcl-2/Bax ratio, to activating caspase-3.[6,7] Furthermore, increased oxidative stress also has an impact on apoptosis in the central nervous system. The results showed that the amount of apoptosis in the brain of zebrafish larvae exposed to 3% glucose at 72 hpf increased drastically compared to the control. This correlates with impaired mitochondrial function and increased intrinsic apoptosis pathways due to ROS exposure.[4] Curcumin, as the main active compound in turmeric, is known to have strong antioxidant and anti-inflammatory properties.[16] Administration of curcumin nanoemulsion, especially at a dose of 0.625 µg/mL, has been shown to significantly reduce the amount of apoptosis in the brain and the rate of embryonic mortality, indicating that curcumin is able to suppress ROS production, repair pancreatic β cells, and increase the expression of protective proteins such as HSP-70 and HO-1.[7]

Not only does it affect the survival rate and apoptosis, hyperglycemia also affects the morphological development and neurological function of zebrafish larvae. One of the morphological manifestations found is a decrease in the size of the larval head (microcephaly), which is caused by oxidative stress during organogenesis.[22] Oxidative stress can interfere with the expression of important genes such as PAX3 and increase the activation of P53, which ultimately triggers the mitochondrial apoptosis pathway through increased Bax and cytochrome C.[22] This condition supports the finding that hyperglycemia is teratogenic to the development of the central nervous system.

In terms of neurological function that can be observed from motoric, the results of motility and locomotor tests show that exposure to 3% glucose causes larvae to have weaker movements than the control group, although all larvae still show a response to stimulation. This is assumed to be the impact of motor neuron damage due to oxidative stress and apoptosis, which causes disruption of nerve impulse transmission to the muscles and reduces muscle contraction strength. Interestingly, administration of curcumin nanoemulsion at a dose of 0.625 µg/mL successfully increased the locomotor ability of larvae, to the same level as the control group, proving its effectiveness in improving neuromuscular disorders.

The highest dose of 1.25 µg/mL actually decreased locomotor ability and insulin levels, indicating a toxic effect on the nervous system. This shows that the effects of curcumin are dose-dependent and too high can actually have the opposite effect. This finding is different from several previous studies using turmeric extract in the form of nanoemulsion, which found that a dose of 1.25 µg/mL was the optimal dose.[23,24] The difference in effects between turmeric

extract nanoemulsion and curcumin nanoemulsion at a dose of 1.25 µg/mL may be caused by turmeric extract containing a mixture of bioactive compounds other than curcumin, such as demethoxycurcumin, bisdemethoxycurcumin, essential oils (turmerone), and other phenolic compounds, while curcumin nanoemulsion contains curcumin in a purer form, which can increase its bioavailability and pharmacological effects, but is also potentially more toxic at high doses.[25] Polyphenols, including curcumin, have antioxidant activity, but at high doses they can actually be pro-oxidants, causing oxidative stress and triggering apoptosis (cell death) [16]. High doses of curcumin affect the neuromuscular system of zebrafish larvae causing decreased motility and locomotory[10]. High doses of curcumin can cause an increase in reactive oxygen species (ROS) leading to mitochondrial dysfunction in muscle and nerve cells[10]. This can reduce the production of ATP needed for muscle contraction and motor activity, causing larvae to become weaker[10]. Curcumin in high doses can also interact with neurotransmitter receptors, such as GABA and glutamate receptors, which play a role in coordinating movement. If glutamate activity (excitatory) is disrupted or there is an increase in GABA (inhibitory), larvae can experience hypoactivity or decreased response to stimuli[10]. This may explain why a dose of 1.25 µg/mL is toxic, while a dose of 0.625 µg/mL still provides a therapeutic effect.

Overall, the results of this study indicate that exposure to 3% glucose causes impaired zebrafish embryo development through oxidative stress and apoptosis mechanisms, which affect embryo morphology, nerve function, and viability. Administration of curcumin nanoemulsion, especially at a dose of 0.625 µg/mL, provides significant protection against these damages through antioxidant, anti-inflammatory, and glucose metabolism improvement mechanisms, making it a potential candidate for supportive therapy in the DMG model[25]. However, high doses of curcumin should be avoided because they can trigger toxic effects on the nervous system and interfere with larval motor function[16,25]. From the results of this study, it is also hoped that there will be further research related to the TD50 and LD50 toxicity tests to determine the safety profile of curcumin nanoemulsion. This study also has limitations in the aspect of the molecular mechanism underlying the effects of curcumin nanoemulsion on brain cell development and locomotor function of zebrafish larvae in a gestational diabetes model. This study has not explored the expression of genes or proteins that play a role in the nervous system, oxidative stress, and relevant cellular signaling pathways. Further research is also needed regarding the mechanism of administration of curcumin nanoemulsion which can affect brain development, locomotor, and mortality of zebrafish with gestational diabetes mellitus models. In addition, further research is also needed regarding the effect of administering nanocurcumin in other dosage forms besides nanoemulsion on zebrafish with gestational diabetes mellitus models to compare their effectiveness as herbal medicines.

5. Conclusion

Based on the results of this study, the administration of curcumin nanoemulsion at an optimal dose of 0.625 µg/ml has been proven to have positive effects on zebrafish (*Danio rerio*) larvae and embryos with a gestational diabetes mellitus (GDM) model. The curcumin nanoemulsion was able to enhance larval locomotor activity without affecting motility, reduce embryo mortality rates, inhibit the reduction of head size in larvae, and decrease the level of apoptosis in embryonic brain tissue. Therefore, curcumin nanoemulsion at this dosage shows potential as an effective herbal therapeutic agent for gestational diabetes mellitus.

However, to strengthen these findings and support the broader application of curcumin nanoemulsion, further studies are needed. These should include toxicity testing (TD50 and LD50) to determine its safety profile, as well as investigations into the molecular mechanisms underlying its effects on motility, locomotor activity, mortality, and brain development. Additional studies with a wider range of doses, particularly between 0.3125 µg/ml and 1.25 µg/ml, are recommended to identify the minimum and maximum effective doses. Comparative research using different curcumin formulations beyond nanoemulsion is also necessary to evaluate relative effectiveness. Furthermore, quantitative analysis of glucose and curcumin metabolism, along with identification of the specific brain regions affected by apoptosis, would provide a deeper understanding of curcumin's mechanism of action in the gestational diabetes mellitus model.

6. Author Contribution

S., M.S., A.O., Q.A., and A.T. carried out the experiment. S. wrote the manuscript with support from M.S., A.O., Q.A., and A.T. collected the data sample. H.W. helped supervise the project and conceived the original idea. N. and I.W. conceived the original idea.

7. Acknowledgements

We would like to thank the Department of Pharmacology and the Department of Biochemistry and Biomedicine, Faculty of Medicine, Brawijaya University for providing the opportunity and facilities during this research.

8. References

1. Adli FK. Diabetes Melitus Gestasional: Diagnosis dan Faktor Risiko. Jurnal Medika Hutama. 2021;3(3):1545-51.

2. International Diabetes Federation. IDF Diabetes Atlas. 10th edn. Brussels, Belgium: 2021.
3. Li M, Song Y, Rawal S, Hinkle SN, Zhu Y, Tekola-Ayele F, et al. Plasma Prolactin and Progesterone Levels and the Risk of Gestational Diabetes: A Prospective and Longitudinal Study in a Multiracial Cohort. Front Endocrinol (Lausanne). 2020;11:1–10.
4. Negrato CA, Marques PR, Leite HB, Torigoe CN, Silva BF, Costa K, et al. Glycemic and nonglycemic mechanisms of congenital malformations in hyperglycemic pregnancies: a narrative review. Arch Endocrinol Metab. 2022;66(6):908-18.
5. Trimardiyah T. Relationship of temporary blood glucose levels during pregnancy and baby's birth weight. Jurnal Ners dan Kebidanan Indonesia. 2021;9(3):233-40.
6. Sari LM. Apoptosis: Mekanisme Molekuler Kematian Sel. Cakradonya Dent J. 2018;10(2):65–70. doi:10.24815/cdj.v10i2.11701.
7. Saucedo R, Ortega-Camarillo C, Ferreira-Hermosillo A, Díaz-Velázquez MF, Meixueiro-Calderón C, Valencia-Ortega J. Role of oxidative stress and inflammation in gestational diabetes mellitus. Antioxidants (Basel). 2023;12(10):1812.
8. Sifuentes-Franco S, Padilla-Tejeda DE, Carrillo-Ibarra S, Miranda-Díaz AG. Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy. Int J Endocrinol. 2018;2018:1875870.
9. Zhao Z, Reece EA. New Concepts In Diabetic Embryopathy. Clin Lab Med. 2013;33(2):207-33.
10. Mustika D. Paparan Glukosa Tinggi Menurunkan Aktivitas Neuron Dopaminergik dan Meningkatkan Apoptosis Sel Otak melalui Jalur Sinyal Estrogen pada Embrio Zebrafish (*Danio rerio*) [dissertation]. Universitas Brawijaya; 2014.
11. Fuchs KDM, Pereira KHNP, Xavier GM, Mendonça JC, Barreto RO, Silva RC, et al. Neonatal hypoglycemia in dogs—pathophysiology, risk factors, diagnosis and treatment. Front Vet Sci. 2024;11:1345933.
12. Nugraha AA. Manajemen Diabetes Melitus Dalam Kehamilan. Jurnal Medika Hutama. 2023 Jul 7;4(04 Juli):3585-601.
13. Masikki MF, Fadhli WM. Farmakologi Kebidanan dalam Masa Kehamilan. NEM; 2022.
14. Proboningsih J, Joeliantina A, Novitasari A, Purnamawati D. Complementary treatment to reduce blood sugar levels of DMT2 patients. Int J Public Health Sci. 2020;9(3):267-71.
15. Fioni, Liena. Manfaat Ekstrak Kunyit dalam Menurunkan Enzim Amilase Pankreas. Unpri Press; [n.d.]
16. Silva PA, Arawwawala LD, Kumari MW, Galappatthy P. Effect of Curcuma longa L. and curcumin on diabetes and its complications: A review. J Ayurvedic Herb Med. 2021;7(2):109-18.
17. Adila Y. Perbandingan Efek Anti-Hiperglikemik Kurkumin Dan Nanopartikel Kurkumin Pada Mencit Jantan Induksi Aloksan Serta Gambaran Histopatologi Pankreas [thesis]. Fakultas Farmasi Universitas Pancasila; 2020.
18. Quispe C, Herrera-Bravo J, Javed Z, Khan K, Raza S, Gulsunoglu-Konuskan Z, et al. Therapeutic applications of curcumin in diabetes: a review and perspective. Biomed Res Int. 2022;2022:Article ID.
19. Nirwan AH. Pembuatan Nanoemulsi Kurkumin dalam Sistem Virgin Coconut Oil (VCO)-Tween 80 dengan Metode Wet Ball Milling [dissertation]. Universitas Brawijaya; 2020.
20. Hardianti M, Yuniarto A, Hasimun P. Zebrafish (*Danio rerio*) Sebagai Model Obesitas dan Diabetes Melitus Tipe 2. JSFK (Jurnal Sains Farmasi & Klinis). 2021;8(2):69-79.
21. Cao Y, Chen Q, Liu Y, Jin L, Peng R. Research Progress on the Construction and Application of a Diabetic Zebrafish Model. Int J Mol Sci. 2023;24(6):5195.
22. Ilham D. Pengaruh Genistein Terhadap Laju Mortalitas Dan Ukuran Kepala Pada Embrio Zebrafish (*Danio rerio*) Yang Diberi Paparan Glukosa Tinggi [dissertation]. Universitas Brawijaya; 2018.
23. Fathir N. Pengaruh Pemberian Nanoemulsi Ekstrak Kunyit Terhadap Panjang Tubuh Larva Zebrafish (*Danio rerio*) Dengan Model Diabetes Gestasional [thesis]. Universitas Brawijaya; 2022.
24. Khotimah, H., Subandi, R., Nastyazka, S. Pengaruh Pemberian Nanoemulsi Ekstrak Kunyit Terhadap Denyut Jantung Larva Zebrafish (Danio rerio) yang Diberi Paparan Glukosa [thesis]. Universitas Brawijaya; 2022.
25. Hassanizadeh, S., Shojaei, M., Bagherniya, M., Orekhov, A.N. and Sahebkar, A., 2023. Effect of nano-curcumin on various diseases: A comprehensive review of clinical trials. Biofactors, 49(3), pp.512-533.
26. Powers, M. A., Cuddihy, R. M., Wesley, D., & Morgan, B., 2010. Continuous glucose monitoring reveals different glycemic responses of moderate-vs high-carbohydrate lunch meals in people with type 2 diabetes. Journal of the American Dietetic Association, 110(12), 1912-1915.
27. Iskandar, Fathnuraini P. Pengaruh Pemberian Nanoemulsi Ekstrak Kunyit Terhadap Apoptosis Pada Mata Larva Zebrafish (Danio Rerio) Yang Diberi Paparan Glukosa. Universitas Brawijaya; 2022.