pISSN:2355-7583 | eISSN:2549-4864
http://ejurnalmalahayati.ac.id/index.php/kesehatan

EFFECT OF CURCUMIN NANOEMULSION ON INSULIN LEVELS AND MALONDIALDEHYDE CONCENTRATION IN ZEBRAFISH EMBRYOS MODEL OF GESTATIONAL DIABETES MELLITUS

Subandi[1*], Galuh Anggarda Paramita[2], Safa'atul Khoir[3], Nurdiana[4], I Wayan Arsana Wiyasa[5]

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

[*)]Email Korespondensi: desobg@ub.ac.id

Abstract: The Effect of Curcumin Nanoemulsion on Insulin Levels and Malondialdehyde concentration in Zebrafish Embryos Model of Gestasional Diabetes. *Gestational Diabetes (GD) is a condition characterized by increased blood sugar levels (hyperglycemia) in pregnant women who were previously undiagnosed with diabetes. Hyperglycemia disrupts the performance of pancreatic β cells that are responsible for producing the hormone insulin. Curcumin functions as an anti-inflammatory, allowing the insulin reaction to occur more quickly and insulin production to increase. Methods. Post Test Only Control Group Design was used in this experimental study which was tested on each research variable consisting of 1000 zebrafish embryos divided into 5 treatment groups with each sample containing 40 embryos, 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). The exposure was carried out at 2-50 hpf and then measured using INS ELISA Kit and MDA ELISA Kit. The results were analyzed using a one-way ANOVA test. Results. NOVA showed p = 0.254 for insulin levels (not significant) and p = 0.029 for MDA concentrations. Post-hoc analysis revealed that the significant difference occurred only between the negative (K−) and positive (K+) control groups (p = 0.012), while the curcumin-treated groups (K1, K2, K3) showed no statistically significant differences compared with either control group (p > 0.05). Conclusion. Curcumin nanoemulsion tended to increase insulin levels and reduce MDA concentrations, but these effects were not statistically significant (p > 0.05). The induction of hyperglycemia with 3% glucose successfully established oxidative stress in zebrafish embryos, validating the model. Further studies with larger sample sizes and molecular analyses are needed to confirm the biological trends observed.*
Keywords: *Curcumin nanoemulsion, Gestasional diabetes, Zebrafish embryos.*

Abstrak: Pengaruh Nanomulasi Kurkumin pada Kadar Insulin dan Malondialdehyde Embrio Zebrafish dengan Diabetes Gestasional. Diabetes Gestasional (DG) adalah kondisi yang ditandai dengan peningkatan kadar gula darah (hiperglikemia) pada wanita hamil yang sebelumnya belum terdiagnosis diabetes. Hiperglikemia mengganggu kinerja sel β pankreas yang bertanggung jawab untuk memproduksi hormon insulin. Kurkumin berfungsi sebagai anti-inflamasi, memungkinkan reaksi insulin terjadi lebih cepat dan produksi insulin meningkat. Desain *Post Test Only Control Group* digunakan dalam studi eksperimental ini yang diuji pada masing-masing variabel penelitian yang terdiri dari 1000 embrio zebrafish yang dibagi menjadi 5 kelompok perlakuan dengan setiap sampel mengandung 40 embrio, terdiri dari kontrol negatif, kontrol positif yang terpapar 3% glukosa, dan kelompok diabetik yang diberikan nanoemulsi kurkumin dengan tiga dosis berbeda (0,3125 μg/ml, 0,625 μg/ml, 1,25 μg/ml). Paparan dilakukan pada 2-50 hpf dan kemudian diukur menggunakan INS ELISA Kit dan MDA ELISA Kit. Hasilnya dianalisis menggunakan uji *One Way ANOVA*. P *value* untuk Uji *One Way* ANOVA p=0,254 untuk kadar insulin dan p=0,029 untuk konsentrasi MDA. Hasil *post hoc Tukey* menunjukkan bahwa perbedaan signifikan hanya terjadi antara kelompok kontrol negatif (K−) dan kontrol positif (K+) ($p = 0,012$), sedangkan kelompok perlakuan (K1, K2, K3) tidak menunjukkan perbedaan yang signifikan dibandingkan kedua kelompok kontrol ($p > 0,05$). Pemberian nanoemulsi kurkumin cenderung meningkatkan kadar insulin dan menurunkan konsentrasi MDA, namun tidak signifikan secara statistik ($p > 0,05$). Induksi hiperglikemia dengan glukosa 3% berhasil menimbulkan stres oksidatif pada embrio ikan zebra, sehingga model ini valid untuk menggambarkan kondisi stres oksidatif akibat hiperglikemia. Penelitian lanjutan dengan jumlah sampel lebih besar dan analisis molekuler diperlukan untuk mengonfirmasi kecenderungan biologis yang ditemukan.

Kata Kunci : Nanoemulsi kurkumin, Diabetes gestasional, Embrio zebrafish.

INTRODUCTION

Gestational diabetes (GD) is a condition of increased blood sugar levels (hyperglycemia) in pregnant women who were previously undiagnosed with diabetes (Pheiffer, et al. 2020). Its prevalence is still quite high at 7% - 11.6% of all pregnancies worldwide, and in Indonesia, there was an increase in 2020 to 3% - 5%, up from only 1.9% - 3.6% in 2019. And there are still many cases of GD that remain undiagnosed (Ilham, et al. 2024). The increase in blood sugar levels in pregnant women is usually detected at more than 20 weeks of gestation, which is when the levels of placental hormones rise that have effects opposite to those of insulin. Generally, women will secrete more insulin to maintain blood sugar levels (Plows, et al. 2018). As a result of excessive insulin secretion, there is hypertrophy and hyperplasia of the β cells of the pancreas, leading to insulin resistance and glucose intolerance. This can hinder the signaling for the process of glucose metabolism, causing insulin synthesis to be disrupted and resulting in insulin deficiency (Adli, 2021).

The condition of gestational diabetes in women increases the risk of complications during pregnancy and childbirth (Kosanto, et al. 2016). The complications that arise include the mother being at high risk of developing preeclampsia, cardiovascular complications, and nephropathy. Several studies indicate that complications of diabetes mellitus are caused by the occurrence of oxidative stress (Pieme, at al. 2017). Oxidative stress is a state of imbalance where the number of free radicals is higher than the number of antioxidants in the body, leading to a cascade of damage starting from cells to higher levels. Oxidative stress is the basis of the pathogenesis of chronic disease processes (Halliwel & Gutteridge, 2007).

Increased oxidative stress plays a crucial role in the etiology and complications of diabetes mellitus (DM). Hyperglycemic conditions stimulate the production of reactive oxygen species (ROS) from various sources and result in weakened antioxidant defenses (Chen, et al. 2024). The pathways for ROS formation in hyperglycemic conditions include the polyol pathway, protein glycation, glucose autoxidation, and lipid autoxidation that accelerate the formation of reactive oxygen compounds (González, et al. 2023). Lipids are one of the main targets of ROS. Peroxyl radicals can remove hydrogen from lipids, generating hydrogen peroxide that increases free radicals (Tiwari, et al. 2013). Lipid peroxidation is the process where polyunsaturated fatty acids in cell membranes react with reactive oxygen species (ROS) such as free radicals. The degree of lipid peroxidation can be indicated by the level of malondialdehyde (MDA), which is the end product of polyunsaturated fatty acid (PUFA) peroxidation (Gohil, et al.

2020). MDA (Malondialdehyde) that forms will be metabolized and react with DNA, cellular proteins, and tissues, resulting in additional products that cause biomolecular damage (Ayala, et al. 2014). An increase in MDA levels indicates an increase in lipid peroxidation and can be used as a biomarker for oxidative stress (Tiwari, et al. 2013).

The main treatment for DG cases is metformin therapy and insulin. The administration of insulin therapy is very complex and requires collaboration among experts in its management. Meanwhile, metformin therapy has not yet been definitively tested for long-term safety. Therefore, traditional medicine derived from plants is being developed, one of which is turmeric containing curcumin. The phenolic compounds found in curcumin can act as hydroxyl radical scavengers and anti-diabetic agents by stimulating adipocyte differentiation and activating PPAR-γ (Proliferator-Activated Receptor Gamma), so ROS (Reactive Oxygen Species) will decompose and insulin sensitivity will significantly increase (Malik, et al. 2021). Curcumin has a mechanism of action on pancreatic beta cells by increasing insulin secretion and reducing apoptosis of pancreatic beta cells, which allows for more pancreatic beta cells to produce insulin (Istriningsih & Solikhati, 2021).

The selection of zebrafish embryos as an experimental animal model is due to their ability to easily adapt to disturbances occurring during the process of embryogenesis, which facilitates embryo manipulation (Kinkel & Prince, 2009). Zebrafish also have a pancreatic function similar to that of humans, particularly in blood sugar homeostasis. The zebrafish pancreas begins to develop as early as 24 hpf (hours post fertilization), and β cells start to secrete insulin from 15 hpf (Singh, 2019).

Previous research by (Cao, et al. 2023) showed a decrease in insulin levels in zebrafish with type 1 and type 2 diabetes models. There was also prior research by Fathir (2022) that discussed the complications of gestational diabetes conditions in zebrafish and its effects after administration of curcumin nanoemulsion, with results showing positive effects after exposure. Therefore, there is a strong possibility that there is a decrease in insulin and MDA levels in zebrafish embryos with a gestational diabetes model, and an increase in insulin levels after administration of a certain dose of curcumin nanoemulsion. The researcher aims to definitively prove the effect of curcumin nanoemulsion administration in increasing insulin levels in zebrafish embryos with a gestational diabetes model.

METHODS

This research was an experimental study using a *Post-Test Only Control Group Design*. A total of 1,000 zebrafish embryos were used, divided into 25 experimental units, each consisting of 40 embryos. The samples were allocated into five treatment groups: a negative control group, a positive control group exposed to 3% glucose, and three diabetic groups treated with curcumin nanoemulsion at doses of 0.3125 μg/mL, 0.625 μg/mL, and 1.25 μg/mL. The treatment was conducted from 2 to 50 hours post-fertilization (hpf), with medium replacement every 24 hours. Insulin levels were measured using the INS ELISA Kit (Fish Insulin) and MDA levels using the MDA ELISA Kit. The absorbance readings were obtained using an ELISA reader and analyzed with the ELISA Standard Curve and Curve Expert software to determine concentration values. The concentration data for insulin and MDA levels were analyzed using IBM SPSS Statistics 30. Normality was tested with the Shapiro–Wilk test, followed by tests for homogeneity and distribution prior to conducting the one-way ANOVA. A p-value < 0.05 was considered statistically significant.

This study received ethical approval from the Research Ethics Committee, Faculty of Medicine, Brawijaya University (No. 367/EC/KEPK-S1-KB/10/2024).

RESULTS

Effect on Insulin Levels

From the research results, it was found that the concentration of zebrafish embryos exposed by 3% glucose for 48 hours experienced a decrease of ±50.21 μg/ml compared to the zebrafish embryos that were only in embryonic medium, thus it can be concluded that this exposure effectively causes the embryos to become gestationally diabetic. There is an increase in the average insulin level in zebrafish embryos with gestational diabetes that were given a curcumin nanoemulsion intervention at doses of 0.3125 μg/ml and 0.625 μg/ml. However, the results from both doses still do not match the insulin levels when no intervention is given. Meanwhile, the curcumin nanoemulsion intervention at 1.25 μg/ml showed a decrease in average insulin levels, even lower than that of gestational diabetes without curcumin nanoemulsion intervention.

Table 1. Results of Insulin Concentration Levels in 50 hpf Zebrafish Embryos (μg/ml)

(N)	K- (Embrionic Medium)	K+ (EM + 3% Glucose)	K1 (EM + 3% Glucose + Curcumin nanoemulsion 0,3125 μg/ml)	K2 (EM + 3% Glucose + Curcumin nanoemulsion 0,625 μg/ml)	K3 (EM + 3% Glucose + Curcumin nanoemulsion 1,25 μg/ml)
1	209.9	135.05	123.14	61.69	125.07
2	113.09	133.53	94.13	157.02	95.84
3	174.52	166.14	141.86	107.11	118.19
4	179.79	33.93	169.40	127.64	110.16
5	139.84	97.47	49.08	164.44	116.34
Mean ± SD	163.43	113.22	115.52	123.58	113.12

Based on the normality test using the Shapiro-Wilk test, the data showed p>0.05, indicating that the data are normally distributed. Furthermore, in the distribution and homogeneity tests, which are prerequisites for the One Way ANOVA test, p>0.05 was also found, allowing us to conclude that the data are normal and homogeneous. Based on the analysis of the One Way ANOVA test on insulin levels, it was found that p>0.05, specifically p=0.254, which means that there is no significant difference or it can be concluded that the treatment given does not affect the results. Therefore, the data indicate that in this study, the most effective dose of curcumin nanoemulsion in increasing insulin levels in zebrafish embryos with gestational diabetes is 0.625 μg/ml. In contrast, the dose of curcumin nanoemulsion at 1.25 μg/ml shows decrease in insulin levels in zebrafish embryos with gestational diabetes.

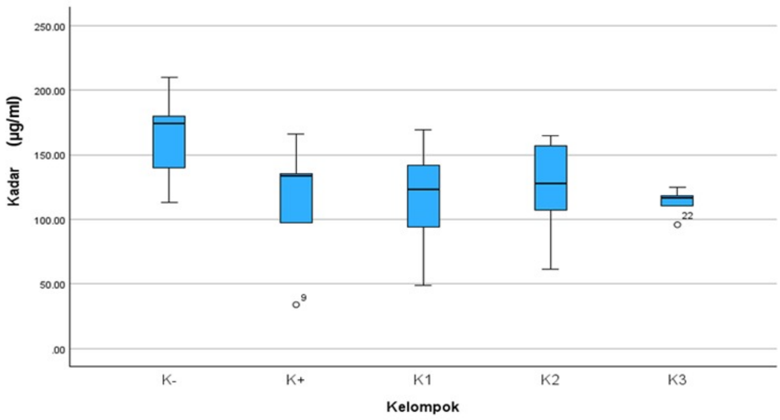

Figure 1. Insulin Concentration Levels in 50 hpf Zebrafish Embryos

This study aimed to evaluate the effect of curcumin nanoemulsion on malondialdehyde (MDA) levels as a biomarker of oxidative stress in glucose-induced zebrafish embryos. The results of MDA concentration measurements are presented in Table 2.

Table 2. MDA Concentration Levels in 50 hpf Zebrafish Embryos (µg/ml)

(N)	K- (Embrionic Medium)	K+ (EM + 3% Glucose)	K1 (EM + 3% Glucose + Curcumin nanoemulsion 0,3125 µg/ml)	K2 (EM + 3% Glucose + Curcumin nanoemulsion 0,625 µg/ml)	K3 (EM + 3% Glucose + Curcumin nanoemulsion 1,25 µg/ml)
1	2.69	8.56	16.19	30.29	6.57
2	3.56	25.16	11.40	18.94	19.44
3	5.92	72.07	23.07	14.01	16.29
4	4.22	42.65	15.24	23.81	8.81
5	1.62	16.96	23.74	7.73	27.93
Mean ± SD	3.60	33.08	17.93	18.96	15.81

One-way ANOVA analysis revealed a significant difference among the groups ($p = 0.029$). Post-hoc Tukey's test indicated that the significant difference occurred only between the negative control (K−) and positive control (K+) groups ($p = 0.012$), while the treatment groups (K1, K2, and K3) showed no statistically significant differences compared to either the negative or positive control groups ($p > 0.05$).

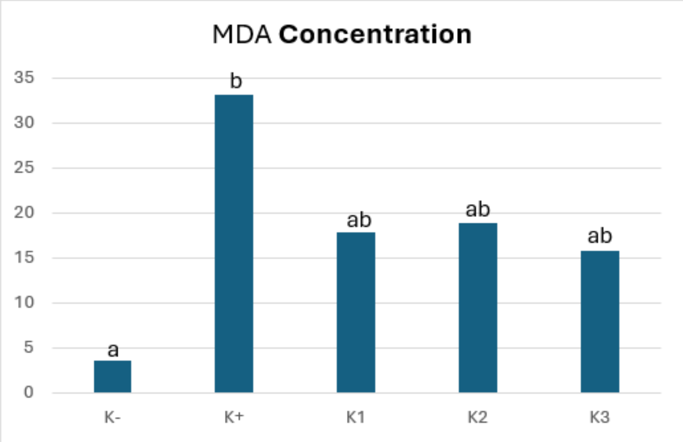

Figure 2. Mean MDA concentration (μg/mL) in zebrafish embryos

Figure 2 illustrates the differences in MDA levels among groups, where different letter notations indicate statistically significant differences ($p < 0.05$), based on Tukey's post hoc test. The positive control group (K+) differed significantly from the negative control group (K−), while the treatment groups (K1, K2, K3) showed no significant differences compared to either control.

DISCUSSION

The Influence of Glucose on Insulin Levels in Zebrafish Embryos

From the research results, it was found that the embryos of zebrafish exposed to 3% glucose for 48 hours experienced a decrease, proving that this exposure effectively causes the embryos to become gestationally diabetic. This occurs because the zebrafish pancreas begins to develop from 24 hours post-fertilization (hpf), and β cells start secreting insulin from 15 hpf (Singh, 2019). This hyperglycemic condition will disrupt the performance of β cells in the pancreas, which are the cells responsible for secreting insulin hormone in the body. Hyperglycemia can also increase TNF-α, exacerbating the formation of Reactive Oxygen Species (ROS). This condition can lead to a decrease in the auto-phosphorylation of insulin receptors, conversion of insulin receptor substrates into inhibitors of insulin receptor tyrosine kinase activity, and a reduction in insulin-sensitive glucose transporter (GLUT-4) (Widowati, 2008).

The Effect of Curcumin Nanoemulsion on Insulin Levels in Embryonic Zebrafish with Gestational Diabetes

The administration of nanoemulsion curcumin to zebrafish embryos with gestational diabetes can elevate insulin levels at several concentrations, but not significantly. This increase is due to the phenolic compounds in curcumin which are useful for scavenging superoxide anions and hydroxyl radicals. The mechanism of action of curcumin as an antidiabetic involves stimulating adipocyte differentiation and exhibiting activity against PPAR-γ (Proliferator-Activated Receptor Gamma). The activated PPAR-γ can inhibit the inflammatory process and minimize cell death risk by decomposing ROS. Activation of PPAR-γ can also enhance insulin sensitivity so that blood sugar levels can be well controlled (Malik, et al. 2021). When blood glucose levels drop, ATP-sensitive K Channels in the β cell membrane will open, allowing potassium ions to leave the β cell, with the aim of maintaining the membrane potential in a hyperpolarized state so that Ca Channels close. As a result, calcium cannot enter the β cell, which decreases the β cell's stimulation for insulin secretion, leading to a reduction in insulin levels (Permana, 2017). The administration of nanoemulsion curcumin at several concentrations in this study has been shown to increase insulin levels in embryonic zebrafish with a gestational diabetes model. The average insulin levels increased in embryonic zebrafish with gestational diabetes that were given nanoemulsion curcumin intervention at concentrations of 0.3125 μg/ml and 0.625 μg/ml.

Compared to zebrafish embryos with gestational diabetes, the increase in insulin levels when given curcumin nanoemulsion at a concentration of 0.3125 μg/ml was approximately ±2.3 μg/ml and at a concentration of 0.625 μg/ml was approximately ±10.36 μg/ml. However, this increase was still not significant. Even at a concentration of 1.25 μg/ml, insulin levels in zebrafish embryos experienced a decrease compared to all variables. This is consistent with research by Andrade et al. (2022) that high concentrations of curcumin can lead to various adverse consequences on the development of zebrafish embryos and severe or mild teratogenic characteristics. This is because high concentrations of curcumin are toxic to cells that induce apoptosis. Turmeric rhizomes contain active compounds that are medicinally beneficial called curcuminoids, which belong to the class of phenolic compounds. In addition to being antioxidant, some secondary metabolites of phenolic compounds can also be pro-oxidant. Excessive levels of flavonoids in the body can be oxidized by peroxidase enzymes to form free radical compounds that can oxidize glutathione in hepatocytes (Putri, et al. 2019).

The Effect of Hyperglycemic Conditions on MDA Levels in Zebrafish Embryos

The significant increase in MDA levels in the positive control group (K+) compared to the negative control group (K−) indicates that 3% glucose induction effectively triggers oxidative stress in zebrafish embryos. This is consistent with a study by Uthaiah et al. (2022), which demonstrated a marked elevation in MDA levels (from 1.82 to 3. μg/ml) in a hyperglycemic zebrafish model. Hyperglycemic conditions stimulate the overproduction of reactive oxygen species (ROS) from multiple sources, weakening endogenous antioxidant defenses and increasing oxidative stress. The main pathways contributing to ROS production under hyperglycemia include the polyol pathway, protein glycation, glucose autoxidation, and lipid peroxidation, which collectively accelerate the generation of reactive oxygen species (Yi, et al. 2023).

Lipid peroxidation is a process in which unsaturated fatty acids in cell membranes react with reactive oxygen species (ROS), such as free radicals. The extent of lipid peroxidation can be indicated by the concentration of malondialdehyde (MDA), a terminal product of polyunsaturated fatty acid (PUFA) peroxidation (Gohil, et al. 2020). The MDA formed is metabolized and reacts with DNA, forming adducts at deoxyguanosine and deoxyadenosine residues and also binds to lysine, histidine, and arginine residues in proteins (Wishart, et al. 2025), thereby disrupting biomolecular functions and triggering cellular damage (Pomi, et al. 2024).Therefore, elevated MDA levels reflect increased lipid peroxidation and are widely used as a biomarker of oxidative stress (Batir, et al. 2025).

The Effect of Curcumin Nanoemulsion on MDA Levels in Zebrafish Embryos with Gestational Diabetes

The administration of curcumin nanoemulsion (K1, K2, K3) resulted in a descriptive but not statistically significant reduction in MDA levels compared to the positive control group (K+). Although a downward trend was observed, the differences among the treatment groups were not significant ($p > 0.05$), indicating that curcumin nanoemulsion did not produce a measurable reduction in oxidative stress within the tested concentrations. The greatest decrease was observed at the dose of 1.25 μg/mL (K3), with a mean MDA concentration of 15.81 μg/mL, suggesting a non-significant biological tendency toward antioxidant protection rather than a confirmed effect. Curcumin is known to exert antioxidant effects by both directly scavenging reactive oxygen species (ROS) and inducing the endogenous antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Singha *et al.*, 2024). Nano-formulations, such as PLGA-based nanoparticles and nanoemulsions, have been shown to reduce ROS production and MDA levels in both zebrafish and in vitro models (Pucci *et al.*, 2024). Chuacharoen and Sabliov (2019) also demonstrated that curcumin nanocarriers, including AuNP-curcumin and curcumin-loaded nanoparticles, enhanced antioxidant activity and reduced MDA levels more effectively than free curcumin. Moreover, curcumin has been reported to activate the Nrf2 transcription pathway, promoting the expression of antioxidant genes such as *SOD* and *GPx*, thus reducing lipid peroxidation (Pucci *et al.*, 2024). These findings align with the present study, in which the K3 group exhibited the most pronounced reduction in MDA (<16 μg/mL); however, this reduction was not statistically significant ($p > 0.05$), indicating a biologically plausible yet inconclusive protective trend.

CONCLUSION

Curcumin nanoemulsion tended to increase insulin levels in zebrafish embryos with a gestational diabetes model; however, this effect was not statistically significant ($p > 0.05$). The induction of hyperglycemia using 3% glucose significantly increased MDA levels, confirming the presence of oxidative stress and validating the model as a reliable representation of glucose-induced oxidative stress. Although the administration of curcumin nanoemulsion (0.3125–1.25 μg/mL) showed a descriptive reduction in MDA levels, this decrease was not statistically significant ($p > 0.05$). These findings suggest that while curcumin nanoemulsion exhibits a potential biological trend toward antioxidant protection, further studies with larger sample sizes, extended exposure durations, and molecular pathway analyses are required to confirm its efficacy and clarify its underlying mechanisms.

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