

Effect of lycopene and metformin combination on phagocytosis, glycemic control, and oxidative stress in rats with type 2 diabetes

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ABSTRACT

BACKGROUND Hyperglycemia and oxidative stress cause phagocytosis dysfunction in patients with diabetes. A combination of lycopene and metformin can reduce oxidative stress and blood glucose. This study aimed to determine the effect of combined lycopene and metformin on phagocytosis function, glycated hemoglobin A1c (HbA1c), nitric oxide (NO), reactive oxygen species (ROS), and advanced glycation end products (AGEs).

METHODS A randomized controlled study was conducted in rats at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, from August to September 2022. 30 rats were divided into control (n = 5) and type 2 diabetes mellitus (T2DM) (n = 25) groups. Rats in the T2DM group were induced by a high-fat diet combined with streptozotocin-nicotinamide. The 25 rats were then divided into five subgroups: 1 ml coconut oil (DM), 250 mg/kg metformin in 1 ml coconut oil (DMet), 250 mg/kg metformin + 10 mg/kg lycopene in 1 ml coconut oil (DML-10), 250 mg/kg metformin + 20 mg/kg lycopene in 1 ml coconut oil (DML-20), and 250 mg/kg metformin + 40 mg/kg lycopene in 1 ml coconut oil (DML-40). Treatments were administered daily for 4 weeks. The macrophage phagocytosis index (PI), HbA1c levels, ROS, NO, and AGEs serum were evaluated.

RESULTS There was a significant difference in the PI, HbA1c, NO, ROS, and AGEs between the groups ($p < 0.001$). The DML-20 and DML-40 groups had significantly increased PI and decreased NO, ROS, and AGEs levels than metformin alone ($p < 0.05$).

CONCLUSIONS Lycopene combined with metformin could improve phagocytosis function, glycemic control, and oxidative stress.

KEYWORDS glycemic control, lycopene, metformin, oxidative stress, phagocytosis

A decreased phagocytic function is commonly experienced by patients with type 2 diabetes mellitus (T2DM).^{1–5} It has previously been shown that phagocytic activity is significantly reduced by 14.53% in patients with T2DM compared with individuals without T2DM.⁵ This decrease in phagocytosis causes an increased risk of infections, which occur at an incidence of 36.33 per 1,000 people per year.⁶ Poor glycemic control results in oxidative stress, which in turn causes decreased

phagocytosis.^{1,3–5,7} However, due to metabolic memory, improving glycemic control does not prevent oxidative stress, which continues to affect phagocytic activity.⁸ Patients with T2DM have higher levels of nitric oxide (NO), reactive oxygen species (ROS), and advanced glycation end products (AGEs) than individuals without T2DM.^{9–11}

Currently, there is no standardized method of improving phagocytic function. Metformin is typically

used as the first-line treatment for patients with T2DM because of its antidiabetic and antioxidant properties.^{12,13} It has been shown to reduce NO and AGE levels by 33.33%.^{14,15} However, as the effect of metformin on oxidative stress is <50%, patients with T2DM remain susceptible to infection. Therefore, additional antioxidants and immunomodulators are needed to maximize the reduction in oxidative stress and improve phagocytic function in patients with T2DM.

Figueiredo et al¹⁵ recommended additional antioxidant therapy to increase the effectiveness of metformin treatment. It has previously been shown that carotenoid lycopene can reduce oxidative stress and improve phagocytic function.^{16,17} Lycopene alone can reduce NO and ROS levels in lipopolysaccharide-stimulated macrophages by 30–90% and AGE levels by 33.3%.^{15,18} It can also improve glycemic status by lowering fasting blood glucose (FBG) and glycated hemoglobin A1c (HbA1c) levels.¹⁹

A combination of lycopene and metformin has been shown to decrease FBG levels and reduce ROS and AGE levels to a greater degree than either metformin or lycopene alone.^{15,20} This study aimed to evaluate the effect of combined lycopene and metformin treatment on the phagocytosis index (PI), and levels of HbA1c, NO, ROS, and AGEs in a rat model of T2DM.

METHODS

This randomized controlled study was conducted in rats at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, from August to September 2022.

Animals and ethics

Healthy male albino Wistar rats weighing 160–200 g were purchased from the Animals Laboratory of the Center for Food and Nutrition Studies and housed in an animal room with individual stainless steel cages, a 12-hour light–dark cycle, a temperature of 24°C ± 2°C, and a relative humidity of 50.00–60.00%. All the rats had *ad libitum* access to water and food. The experimental procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine of Universitas Diponegoro (No. 28/EC/H/FK-UNDIP/IV/2022). All animals were cared according to the Animal Laboratory Guidelines of the Center for Food and Nutrition Studies.

Experimental design

Thirty Wistar rats were randomly divided into control (n = 5) and T2DM (n = 25) groups. Rats in the T2DM group were fed a high-fat diet containing Comfeed Par S (Japfa Comfeed Indonesia, Indonesia; 60.00%), flour (27.80%), cholesterol (2.00%), folic acid (0.20%), and lard (10.00%). After 2 weeks, rats in the T2DM group were intraperitoneally injected with 45 mg/kg streptozotocin (Sigma-Aldrich, USA) and 110 mg/kg nicotinamide (Nacalai Tesque, Inc., Japan) in citrate buffer (pH 4.6). T2DM was diagnosed after 72 hours of FBG levels of 200 mg/dl.²¹ The 25 rats in the T2DM group were divided into five subgroups, which received the following treatment: 1 ml coconut oil (DM), 250 mg/kg metformin in 1 ml coconut oil (DMet), 250 mg/kg metformin + 10 mg/kg lycopene in 1 ml coconut oil (DML-10), 250 mg/kg metformin + 20 mg/kg lycopene in 1 ml coconut oil (DML-20), and 250 mg/kg metformin + 40 mg/kg lycopene in 1 ml coconut oil (DML-40).

Metformin hydrochloride (99.60%) (PT Phapros Tbk, Indonesia) and tomato extract powder containing 98% lycopene (Sigma-Aldrich) were used in this study. Treatment was administered daily for 4 weeks via oral gavage. The doses of lycopene and metformin were chosen according to previous studies by Eze et al²² and Figueiredo et al,¹⁵ respectively.

Biochemical assays

Four weeks following the last intervention and after an overnight fast, all rats were sacrificed under ketamine anesthesia. Blood samples were collected immediately from the retro-orbital plexus using a capillary glass tube. Blood was allowed to clot, and serum was separated by centrifugation at 3,500 rpm for 10 min. Serum HbA1c, NO, and AGE levels were quantified using rat HbA1c, NO, and AGE FineTest enzyme-linked immunosorbent assay kits (Wuhan Fine Biotech Co., Ltd., China) according to the manufacturer's instructions. Serum ROS levels were quantified following the procedure described by Tang et al²³ with thiobarbituric acid reactive substances (Animals Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia) using the colorimetric method and a spectrophotometer set at a wavelength of 532 nm.

Isolation and culture of peritoneal macrophages

The abdominal skin of each rat was disinfected with 70% alcohol and cut open. Next, 10 ml cold Roswell

Park Memorial Institute (RPMI) medium was injected into the peritoneal cavity, and the same syringe was used to aspirate the peritoneal fluid. The fluid was centrifuged at 1,200 rpm for 10 min, following which the supernatant was discarded, and the cells were resuspended in 3 ml RPMI medium. The cells were counted using a hemocytometer and adjusted to obtain a cell suspension with 2.5×10^6 /ml density. The cell suspension was cultured in a microplate with round coverslips, with 200 μ l (5×10^5 cells) per coverslip. The cells were incubated in a 5.00% carbon dioxide (CO_2) incubator at 37°C for 24 hours.²⁴

PI testing

Following the culture for 24 hours, the medium was removed using a pipette, and the cells were washed twice with RPMI. Latex beads resuspended in RPMI were added to each well in volumes up to 200 μ l, and the cells were further incubated in 5% CO_2 at 37°C for 1 hour, after which cells were washed three times with phosphate-buffered saline, dried $20\text{--}25^\circ\text{C}$, and fixed with methanol for 30 min. The methanol was then removed, and the coverslip was left to dry and then stained with Giemsa 20.00% (v/v) for 20 min, washed with distilled water, removed from the culture wells, and dried at $20\text{--}25^\circ\text{C}$. The number of macrophages

that had phagocytosed latex beads and the number of latex beads within macrophages was calculated under a light microscope at $400\times$ magnification following a previously described procedure.²⁴

Statistical analysis

Data are expressed as mean (standard error of the mean). Differences between groups were analyzed by one-way analysis of variance followed by the least significant difference test. Statistical analyses were performed using SPSS software version 23 (IBM Corp., USA). Differences between mean values were considered significant at $p < 0.05$.

RESULTS

Glycemic control

Figure 1a shows the serum HbA1c levels after 4 weeks of treatment. There was a significant decrease ($p < 0.001$) in serum HbA1c levels, both in the rats with diabetes treated with metformin alone and in those treated with metformin combined with lycopene, compared with the diabetic control group.

The combination of metformin and lycopene exerted a significant effect at all doses compared with the DM group. The effect was dose-dependent (Table 1),

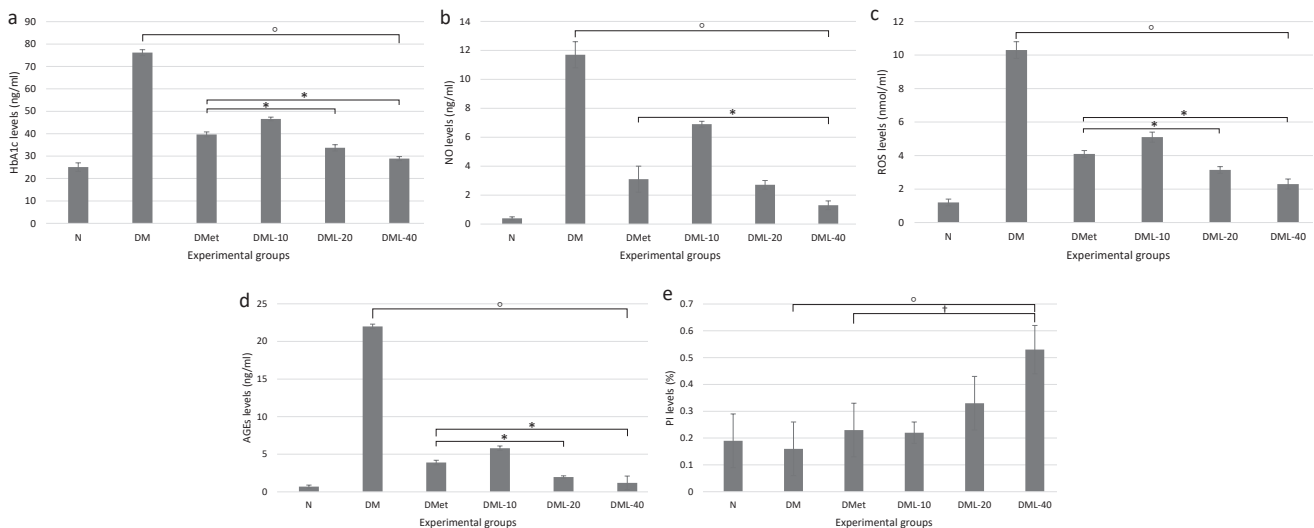


Figure 1. Effect of 4 weeks of lycopene and metformin combination on HbA1c (a), NO (b), ROS (c), AGEs (d), and PI (e) in rats with T2DM. Values are expressed in terms of mean (SEM). All variables showed significantly different results ($p < 0.05$) between the DM group and those treated with metformin alone and those with metformin combined with lycopene (shown in circle). Differences in HbA1c, NO, ROS, and AGEs levels between groups were analyzed using one-way ANOVA followed by the LSD test: $*p < 0.05$; PI levels using Kruskal-Wallis test followed by the Mann-Whitney test: $^{\circ}p < 0.05$. AGEs=advanced glycation end products; ANOVA=analysis of variance; DM=rats with T2DM treated with coconut oil; DMet=250 mg/kg metformin in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; LSD=least significant difference; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; SEM=standard error of the mean; T2DM=type 2 diabetes mellitus

Table 1. Inferential analysis on HbA1c, NO, ROS, AGEs, and PI levels following lycopene and metformin administration in rats with T2DM

| Item | HbA1c* (ng/ml) | NO* (ng/ml) | ROS* (mmol/ml) | AGEs* (ng/ml) | PI† (%) |
|-------------------|----------------|---------------------------|----------------|----------------|---------------------------|
| N vs. DM | -50.68 (0.818) | -11.13 (0.269) | -9.04 (0.183) | -21.19 (0.151) | 4.1 (5.54) |
| DMet vs. DML-10 | -8.91 (0.818) | -3.14 (0.269) | -1.08 (0.183) | -1.93 (0.151) | -0.5 (5.54) |
| DMet vs. DML-20 | 5.25 (0.818) | 0.46 (0.269) [‡] | 0.99 (0.183) | 1.89 (0.151) | -7.7 (5.54) |
| DMet vs. DML-40 | 10.55 (0.818) | 1.74 (0.269) | 1.77 (0.183) | 2.63 (0.151) | -14.5 (5.54) [§] |
| DML-10 vs. DML-20 | 12.16 (0.818) | 3.60 (0.269) | 2.07 (0.183) | 3.81 (0.151) | -7.2 (5.54) |
| DML-10 vs. DML-40 | 17.46 (0.818) | 4.89 (0.269) | 2.85 (0.183) | 4.55 (0.151) | -14 (5.54) [¶] |
| DML-20 vs. DML-40 | 5.3 (0.818) | 1.29 (0.269) | 0.78 (0.183) | 0.74 (0.151) | -6.8 (5.54) |

AGEs=advanced glycation end products; ANOVA=analysis of variance; DM=rats with T2DM treated with coconut oil; DMet=metformin 250 mg/kg in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; LSD=least significant difference; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; SEM=standard error of the mean; T2DM=type 2 diabetes mellitus

Values are expressed in terms of mean (SEM), n = 5

Differences between groups were analyzed using one-way ANOVA followed by the LSD test, * $p < 0.001$, [‡] $p = 0.101$; [§]Kruskal-Wallis test followed by the Mann-Whitney test: [¶] $p < 0.01$, [¶] $p < 0.05$

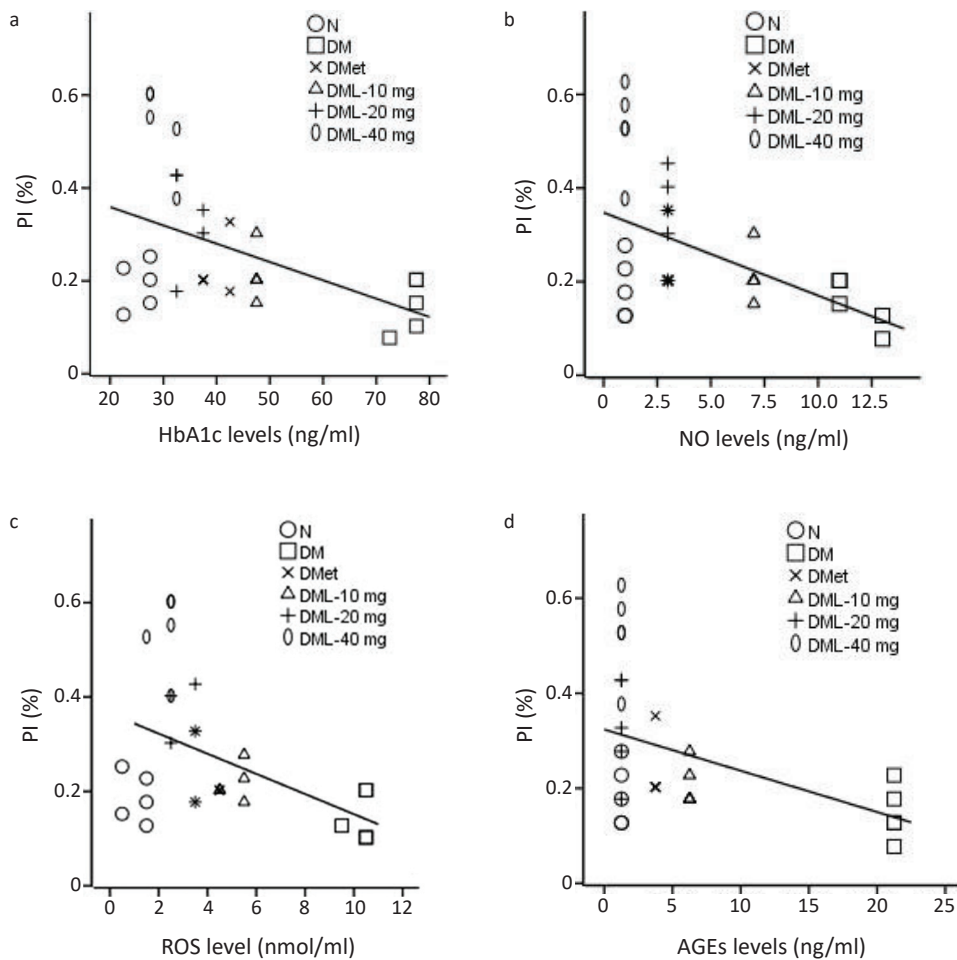


Figure 2. Linear regression of HbA1c levels (a), NO (b), ROS (c), and AGEs (d) with PI in rats with T2DM. AGEs=advanced glycation end products; DM=rats with T2DM treated with coconut oil; DMet=metformin 250 mg/kg in coconut oil; DML-10=250 mg/kg metformin + 10 mg/kg lycopene in coconut oil; DML-20=250 mg/kg metformin + 20 mg/kg lycopene in coconut oil; DML-40=250 mg/kg metformin + 40 mg/kg lycopene in coconut oil; HbA1c=glycated hemoglobin A1c; N=normal rats treated with coconut oil; NO=nitric oxide; PI=phagocytosis index; ROS=reactive oxygen species; T2DM=type 2 diabetes mellitus

and the greatest decrease was observed in the DML-40 group. HbA1c levels in the DML-20 and DML-40 groups were significantly lower than those in the DMet group, with mean differences of 5.25 and 10.55 ng/ml, respectively.

Oxidative stress

The levels of NO, ROS, and AGEs after 4 weeks of treatment are shown in Figure 1b–d. All treatment groups showed significant differences in oxidative stress ($p < 0.001$). The DMet group showed a significant decrease in NO, ROS, and AGE levels compared with the DM group ($p < 0.001$). The combination of metformin and lycopene treatment had a significant effect at all doses compared with the DM group. The effect was dose-dependent (Table 1), and the greatest decrease was observed in the DML-40 group.

PI

The PI of the macrophages after 4 weeks of treatment is shown in Figure 1e. The PI was significantly different in all experimental groups ($p = 0.01$). Metformin administration was associated with a slightly increased PI compared with the DM group. However, rats treated with metformin and 20 or 40 mg/kg lycopene showed significantly increased PI in macrophages ($p < 0.05$). The effect of lycopene was dose-dependent, and the DML-40 group showed the greatest improvement in phagocytic function compared with the DMet group (Table 1).

Rats with lower HbA1c levels had a higher macrophage PI ($r = -0.456$) (Figure 2a). A combination of metformin and lycopene improved phagocytosis by lowering blood glucose levels in T2DM rats. Rats with lower oxidative stress levels had a higher macrophage PI, with r values for ROS, NO, and AGE levels of -0.423 , -0.446 , and -0.440 , respectively (Figure 2b–d). Therefore, combining metformin and lycopene may improve phagocytosis by reducing oxidative stress in rats with T2DM.

DISCUSSION

This study found higher HbA1c, NO, ROS, and AGE levels and a lower PI in rats with diabetes compared with the control. In terms of improving phagocytic function, treatment with a combination of 20 or 40 mg/kg lycopene and metformin was superior to metformin alone. Lycopene can act as an immunomodulator at

the cellular level and increase phagocytosis. The results of the present study are in accordance with those obtained by Durairajanayagam et al,²⁵ who showed that the administration of the antioxidant lycopene increased phagocytosis by reducing oxidative stress and through non-oxidative pathways by effects on the immune system.

Metformin is a hypoglycemic therapy as a first-line treatment option for patients with T2DM.¹² In line with previous studies,^{13–15,20} the present study found lower HbA1c, NO, ROS, and AGE levels in diabetic rats treated with metformin, compared with the DM group. Moreover, the decrease in these levels was greater in rats treated with a combination of metformin and 20 or 40 mg/kg lycopene than in those treated with metformin alone, indicating that this combination was effective in improving HbA1c levels and reducing oxidative stress. However, a previous study revealed that a lower lycopene dosage effectively decreased AGE levels compared with the present study. Following the recommended daily dosage of 20 mg lycopene,²⁶ this study found that a combination of metformin and lycopene at 20 mg/kg was the minimum dose required to reduce oxidative stress and improve phagocytic function; treatment with 40 mg/kg lycopene further improved these results.

This study was limited by the fact that it was conducted only in rats. Additionally, the experiments were carried out only at the end of the intervention period (post-test only); thus, changes could not be observed in real-time. Therefore, further studies with a pre-post-test design are required to confirm the effect of the metformin and lycopene combination in humans.

In conclusion, the combination of lycopene and metformin treatment improved phagocytic function and decreased HbA1c, NO, ROS, and AGE levels in rats with diabetes. The addition of lycopene to metformin treatment regimens might increase the effectiveness of metformin in improving glycemic control, oxidative stress, and phagocytosis.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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