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Lycopene Improves the Metformin Effects on Blood Glucose and Neutrophil Counts in Type 2 Diabetic Rats

Medina Sianturi[1], Neni Susilaningsih[2], Heri Nugroho[3,4], Maria Suryani[1]

[1]Department of Nursing, STIKes Elisabeth, Semarang, Central Java, Indonesia

[2]Department of Histology, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

[3]Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

[4]Department of Interbnal Medicine, Kariadi Teaching Hospital, Semarang, Central Java, Indonesia

Correspondence:
Medina Sianturi,
STIKes Elisabeth, Kawi Street 11,
Semarang, Indonesia
Zip Code: 50252

Email: challenia@gmail.com

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Abstract

In patients with type 2 diabetes mellitus (T2DM), both innate and acquired immunity are weakened by hyperglycemia. Lycopene is one of the hydrocarbon carotenoids that has been widely studied for its powerful antioxidant and anti-inflammatory properties, furthermore act as hypoglycemic and immunomodulator. Herein, we investigated the effect of lycopene and metformin combination on fasting blood glucose (FBG) and neutrophil counts. The rats were divided randomly into six groups, each containing five rats. Group 1 consisted of normal rats (N) and group 2, T2DM (DM) rats, which were administered 0.5 mL of coconut oil; group 3 T2DM rats were administered 250 mg/kg of metformin in 0.5 mL of coconut oil; groups 4,5 and 6 rats were administered a combination of metformin 250 mg/kg with 10 mg/kg (DML-10), 20 mg/kg (DML-20) and 40 mg/kg (DML-40) of lycopene in 0.5 mL of coconut oil, respectively. Treatment was administered every day for 28 days. A model of T2DM rats was induced by a high-fat diet for two weeks combined with streptozotocin–nicotinamide. Data were analyzed with a one-way ANOVA test followed by the least significant difference (LSD) test. There were significant differences in FBG levels and the number of neutrophils in all groups. Lycopene combined with metformin had lower FBG concentrations and higher neutrophil counts compared to metformin monotherapy ($p<0.001$), and these observations were dose-dependent. Lycopene combined with metformin can improve blood glucose and neutrophil counts in rats with diabetes. The highest effect was observed in combination with lycopene at a dose of 40 mg/kg and metformin at a dose of 250 mg/kg.

Keywords

Blood Glucose, Lycopene, Metformin, Neutrophil, T2DM.

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INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) experience disorders of blood glucose metabolism that affect immunity. It has been found that diabetes mellitus (DM) patients experience disturbances in the number and function of neutrophils (1–4). Research on neutrophil counts in DM patients is still controversial. A previous study showed an increase in the number of neutrophils in T2DM patients. It was found that the number of neutrophils with DM was 1.25 times higher than that of those without DM (5,6). The number of neutrophils was found to be higher in females (7), which differed from the study by Eze *et al*. (1), which reported a decrease in neutrophil counts in DM (1). No correlation was found between fasting blood glucose and neutrophil counts (6). Neutrophils are cells of the innate immune system that are the first to arrive at the site of infection and primarily kill microorganisms (5). Neutrophil count is impaired due to decreased neutrophil production and migration (8), which increases the risk of infection and thus the morbidity and mortality of DM patients (9).

The results of studies that have been conducted so far suggest that the use of lycopene as a single therapy can improve glycemic status by reducing fasting blood glucose by 30 - 40% (10,11) and HbA1c by 41% (12), improving insulin resistance levels, and increasing insulin sensitivity (11). Lycopene was also able to reduce the neutrophil counts, although this was not significant, and the neutrophil-lymphocyte ratio (1).

Lycopene is a carotenoid ($C_{40}H_{56}$) found in red fruits and vegetables, including tomatoes, papaya, red peppers and watermelon, that has been made into an extract. The role of lycopene as a nutraceutical has been widely studied. Lycopene treatment is given either in single doses or in combination with other therapies such as metformin and statins. The results showed that lycopene is very beneficial for diabetic patients who are intolerant to statins (13). The combination of metformin with low doses of lycopene can reduce fasting blood glucose levels after two weeks of intervention (14), while high doses of 45 mg/kg can reduce blood glucose by 50%, and the results are the same as with single or combined doses of metformin and lycopene (15). Treatment with lycopene can reduce neutrophil counts, but it is no different from treatment with a different dose of lycopene (1). This is the first study to evaluate the effects of combination therapy with lycopene and metformin on neutrophil counts in rats with T2DM at different doses of lycopene. The aim of this study was to evaluate the synergy of lycopene with metformin and its effects on blood glucose levels and rat immunity against type 2 DM induced by a combination of a high-fat diet, streptozotocin, and nicotinamide.

MATERIALS AND METHODS

Materials

Thirty male albinos Wistar rats 6 weeks old and weighing approximately 160–200 g was purchased from the Laboratory Animals of the Center for Food and Nutrition Studies (CNFS), Gadjah Mada University in Yogyakarta, Indonesia. They were housed in an animal room with a 12-hour light–dark cycle, a temperature of 24ºC ± 2ºC, and a relative humidity of 50%–60%. The rats were housed in individual stainless-steel cages and had ad libitum access to water and food, in accordance with the animal laboratory guidelines of the CNFS (Center for Natural Sciences and Fisheries) at Gadjah Mada University.

Metformin in the form of metformin hydrochloride (99.6%) (Phapros Tbk, Indonesia) and tomato extract powder containing 98% lycopene from Sigma-Aldrich (St. Louis, MO, USA) were used. All groups of rats with and without diabetes were treated with a single dose by gavage daily for 28 days. Doses of lycopene and metformin were chosen according to Eze *et al.* (1) and Figueiredo *et al.* (15), respectively.

The research was carried out in August–September 2022 in the Laboratory Animals of CNFS, Gadjah Mada University. The experimental procedures approved by the local ethics committee of the Medical Faculty of Diponegoro University (approval no. 28/EC/H/FK-UNDIP/IV/2022) and were performed in accordance with the principles expressed in the Declaration of Helsinki. All subjects were cared for according to the animal laboratory guidelines of the CNFS, Gadjah Mada University.

Experimental Design

This study used a completely randomized experimental design. Wistar rats were randomly divided into a normal control group (N group, n = 5) and a type 2 diabetic rats' group (n = 25), which were fed a high-fat diet (Comfeed PAR-s 60%, Flour 27.8%, Cholesterol 2%, Folic Acid 0.2%, and Lard 10%). After two weeks, the type 2 diabetic rats' group were injected with Streptozotocin–Nicotinamide (Nacalai Tesque, Japan) at 45 mg/kg/BW and 110 mg/kg, respectively, in citrate buffer (pH 4.6) intraperitoneally. After 72 h, fasting blood glucose ≥ 200 mg/dl was determined in type 2 diabetic rats (16).

In total, thirty Wistar rats were divided into six groups for treatment with a combination of metformin and lycopene. The first and second groups were normal (N) and type 2 diabetic rats (DM) given 1 mL of coconut oil (CNFS). The third group was type 2 diabetic rats treated with a combination of metformin 250 mg/kg in coconut oil (DMet). The four groups were type 2 diabetic rats treated with a combination of metformin and lycopene with a dose of 10 mg/kg lycopene in 1 mL of coconut oil (DML-10). The fifth group were

type 2 diabetic rats treated with a combination of metformin and lycopene with a dose of 20 mg/kg lycopene in 1 mL of coconut oil (DML-20). The sixth group were type 2 diabetic rats treated with a combination of metformin and lycopene at a dose of 40 mg/kg lycopene in 1 mL of coconut oil (DML-40). All treatments were administered every day for 28 days through an oral feeding tube. Coconut oil can increase the bioavailability of lycopene but does not influence blood glucose concentration (17).

Biochemical Assays

After 28 days of final intervention and overnight fasting, all experimental animals were euthanized under ketamine anesthesia. Blood samples were collected from the retro-orbital flexure using a glass capillary, placed in a tube containing EDTA for neutrophil count, and allowed to clot. Serum was separated by centrifugation at 3,500 rpm (2,000 g) for 10 minutes for the blood glucose exam. Blood glucose concentration was determined by the glucose oxidase method using a Dyasis reagent kit (Holzheim, Germany) following the manufacturer's instructions (18). Neutrophil counts of the rats were determined using an automated analyzer, following the manufacturer's manual for *Hematology Analyzer* Sysmex XP-100 (19).

Statistical analysis

Results were expressed as the mean ± standard deviation. Differences between groups were analyzed using *one-way analysis* of variance followed by the Least Significant Difference (LSD) test. Statistical analyzes were performed with SPSS version 23. Significant differences between mean values were considered at $p < 0.05$.

RESULTS

Fasting Blood Glucose

Figure 1A shows the levels of fasting blood glucose concentrations in the experimental groups after 28 days of treatment. There was a significant difference ($p<0.001$) in FBG in all groups. There was a less significant effect of the combination of metformin with lycopene activity at doses of 20 and 40 mg/kg compared to metformin monotherapy. The mean differences were 16.63 and 23.45 mg/dL, respectively. FBG levels in the combination of metformin and lycopene dosed at 10 mg/kg were lower than those in the metformin monotherapy group, with a mean difference of -16.25 (Figure 1B). There was a strong negative correlation between FBG concentration and neutrophil counts (Figure 2).

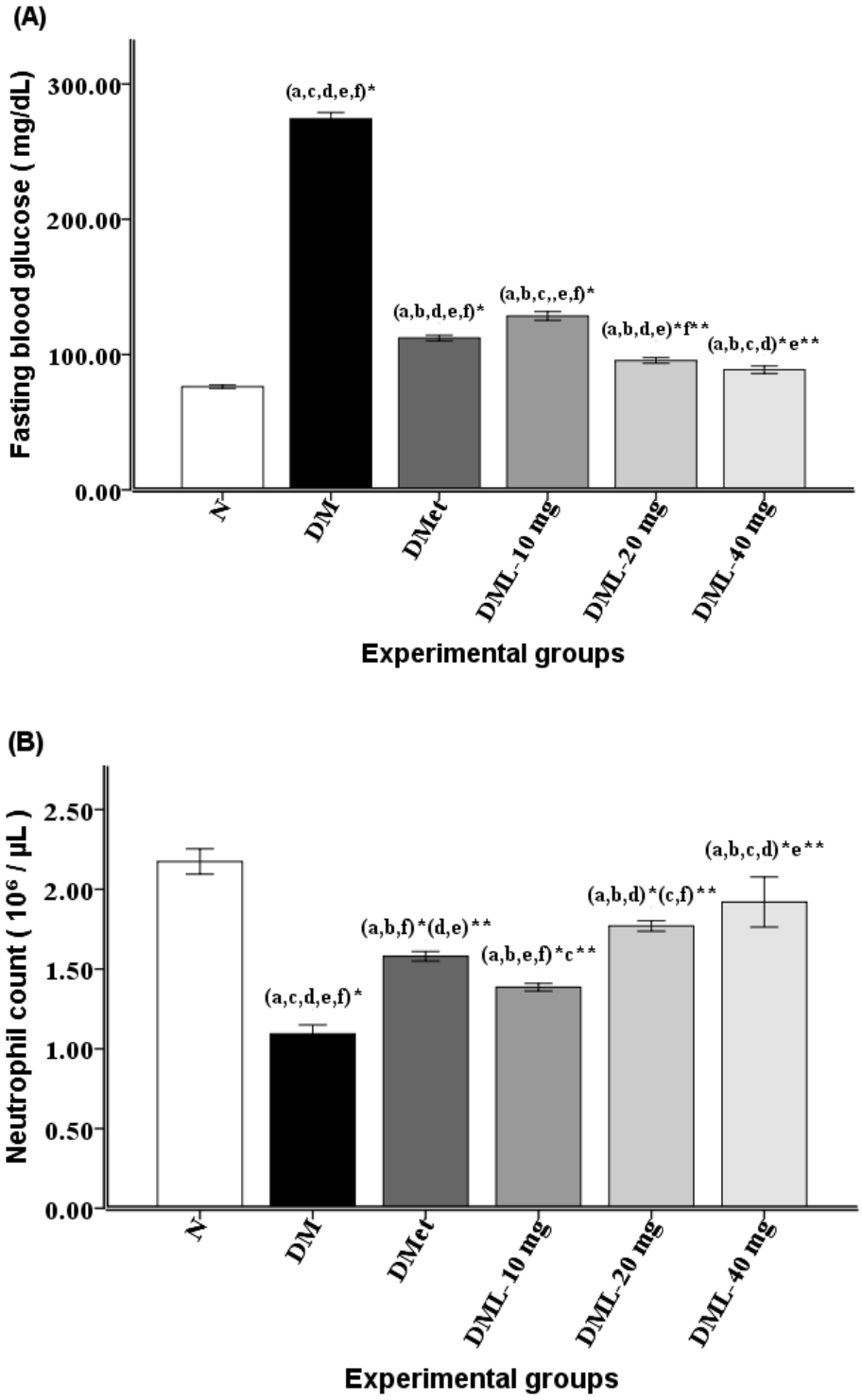

Figure 1. FBG concentration and neutrophil counts in rats with T2DM. Values are expressed in terms of the *mean ± standard error of the mean*. Differences between groups were analyzed using a one-way analysis of variance followed by the least significant difference (*LSD*) test, **p*<0,001, ***p*<0,05 compared to [a]N group; compared to [b]DM group: compared to [c]DMet group; compared to [d]DML-10 group; compared to [d]DML-20 group; and compared to [d]DML-40 group.

Neutrophil Counts

The number of neutrophils in the experimental groups after 28 days of treatment is shown in Figure 1. There was a significant difference in the number of neutrophils between all groups ($p < 0.001$). The combination of metformin and lycopene activity had a greater significant effect at doses of 20 and 40 mg/kg compared to metformin monotherapy. The mean differences were 0.19 and -0.34 x 10^6/µL, respectively. Neutrophil count levels in the combination of metformin and lycopene dosed at 10 mg/kg were higher than those in the metformin monotherapy group, with a mean difference of 0.19 x 10^6/µL (Fiure 1B). There was a significant and strong negative relationship between FBG concentration and neutrophil count (Figure 2).

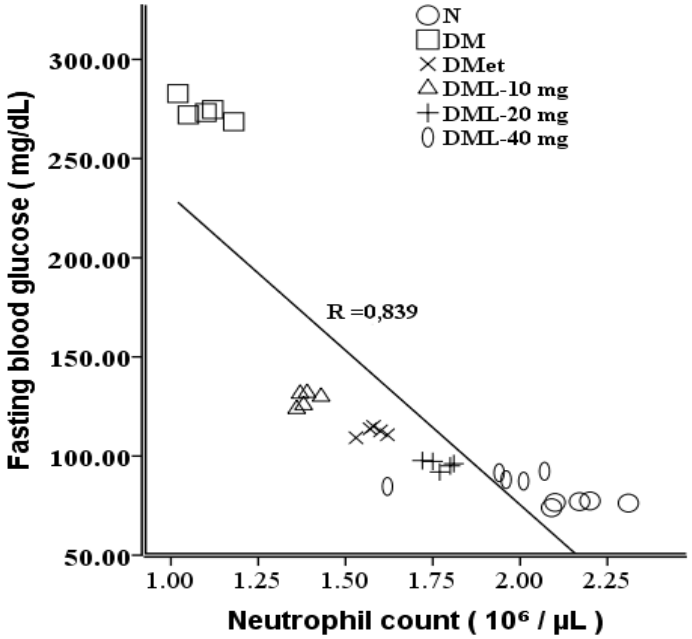

Figure 2. Correlation of FBG and neutrophil counts in rats with T2DM

DISCUSSION

The findings indicate that the induction of T2DM in rats leads to disruptions in glucose metabolism and immune function, as evidenced by increased blood glucose and decreased neutrophil levels. However, administering metformin monotherapy or in combination with lycopene resulted in a decrease in blood glucose and an increase in neutrophil counts, indicating that these treatments effectively improve glucose control and immune function in T2DM rats.

Metformin is the first-line therapy as an oral hypoglycemic in T2DM (20,21). Treatment with metformin monotherapy has been able to reduce blood glucose levels. Metformin lowers blood glucose by

inhibiting glycogenesis in the liver, which results in the reduction of hepatic glucose and an increase in the hepatic cytosolic redox state (22). Administration of metformin and lycopene combination therapy with different doses resulted in a better lowering of blood glucose when compared to metformin monotherapy. These results are in accordance with the research of Haribabau *et al.*, (10); Figueiredo, *et al.*, (11); Yin *et al*., (14); and Zheng, *et al.*, (15); which stated that lycopene, as an antioxidant, can act as an anti-diabetic. Lycopene, as an antidiabetic, can regulate glycolipid metabolism, preventing insulin resistance, inflammation, and fat accumulation (11). It increases insulin sensitivity through lycopene, and increases insulin sensitivity through upregulation of insulin receptors and IGF-1 receptors, PI3K, and the expression of Akt proteins phosphorylated in the hippocampus and cerebral cortex of insulin-resistant rats.

Treatment with a combination of lycopene and metformin resulted in a decrease in fasting blood glucose that was 13-27% higher than a single dose of lycopene (10,11). The addition of lycopene at doses of 20 mg/kg and 40 mg/kg in metformin therapy provided better blood glucose reduction results than the Figueiredo (15) study, where the addition of 45 mg/kg lycopene had the same blood glucose reduction results as metformin monotherapy.

This is the first study to evaluate the effects of combination therapy with lycopene and metformin on neutrophil counts in rats with T2DM. This study showed that treatment with a combination of lycopene and metformin increased neutrophil counts by 53 – 67,6%. This result is different from the studies of Bathia *at al*., (23) and Maldovan *et al*.,(24) which found a decrease in neutrophil count by administering a single dose of lycopene to patients with hepatocellular carcinoma.

These results are consistent with research by Eze *et al.*, (1) which showed that giving lycopene monotherapy to diabetic rats could increase the number of neutrophils, although it was not significantly higher when compared to this study. The results of this study prove that the combination of lycopene and metformin is effective in increasing neutrophil counts in T2DM rats.

The mechanism of action of lycopene can improve the immune status of patients with DM, because lycopene is immunomodulatory and increases the number of neutrophils (1,25). Neutrophils can improve immunity by reducing infections, which are common in patients with T2DM.

The combination of metformin and lycopene can improve the mechanism of neutrophil production and recruitment, causing the number of neutrophils to be higher than with metformin treatment monotherapy. This improvement occurs

through the mechanism of action of lycopene through non-oxidative pathways that can improve immune status (26). In contrast to the study by *Huang et al*., (6) reducing FGD increased the number of neutrophils, proving a relationship between blood glucose levels and the immunity of T2DM patients. This suggests that with a decrease in fasting blood glucose concentration, there is an increase in neutrophil counts.

The increasing doses of lycopene that improved blood glucose and neutrophil count more effectively than metformin monotherapy were 20 and 40 mg/kg. This indicates that the best doses to improve blood sugar and immunity start at 20 mg/kg, which is consistent with the study by Imran et al. (27), that the most effective and recommended dose is 20 mg/kg. The results of this observation prove that lycopene synergizes with metformin in decreasing blood glucose and improving the immune system by increasing neutrophil counts in DM rats, and is expected to help type 2 DM patients (20). The results of this study can be used to support the effectiveness of antioxidants, especially lycopene, in patients with type 2 diabetes and be considered when providing antioxidant based nutritional supplements.

CONCLUSIONS

In a type 2 diabetes mellitus rat model, the combination of lycopene and metformin was founding to lower blood glucose concentrations and increase neutrophil counts. Additionally, lycopene was founding to improve the performance of metformin in lowering blood glucose concentrations and increasing neutrophil counts. The dose-dependent effect of the lycopene and metformin combination was observed. The combination of lycopene at a dose of 40 mg/kg and metformin at a dose of 250 mg/kg has the highest effect.

AUTHOR CONTRIBUTIONS

Medina Sianturi: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing original draft, writing review and editing, and project administration. Neni Susilaningsih: conceptualization, methodology, validation, formal analysis, review, and editing. Heri Nugroho: conceptualization, methodology, validation, review, and editing. Maria Suryani: conceptualization, review, and editing.

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CONFLICT OF INTEREST

The author declars there is no conflict of interest.

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