RESEARCH ARTICLE

Lycopene Enhances the Beta Cell Capacity and Antihyperlipidemic Effects of Metformin on Type 2 Diabetic Rats

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Abstract

BACKGROUND: Hyperglycemia causes dyslipidemia in type 2 diabetes mellitus (T2DM). Metformin monotherapy is known to be less effective at improving glycemic status, insulin function, and lipid profiles. Lycopene is a potential antioxidant and has been shown to be hypoglycemic and hypocholesterolemic. However, the effects lycopene and metformin combination are still up for debate. This study was conducted to determine the potential of lycopene in enhancing the ability of metformin to improve glycemic status, insulin resistance, beta cell capacity, and lipid profile of T2DM rats.
METHODS: Thirty male Wistar rats were randomly divided into six groups: control (N), T2DM-untreated (D), T2DM + metformin (DM), T2DM + metformin + 10 mg/kgBW lycopene (DMLy-10), T2DM + metformin + 20 mg/kgBW lycopene (DMLy-20), and T2DM + metformin + 40 mg/kgBW lycopene (DMLy-40). The treatment was administered once daily through oral route and lasted for 28 days, before blood samples were collected. Fasting blood glucose (FBG) was assessed by oxidase-peroxidase method, fasting serum insulin and HbA1c were measured using enzyme-linked immunosorbent assay (ELISA), while lipid profile was determined using enzymatic methods. The homeostatic model assessment for insulin resistance (Homa-IR) as well as the homeostatic model evaluation of β-cell function (Homa-B) were then calculated.
RESULTS: Fasting serum insulin levels increased significantly (p<10.05) in the DMLy-20 and DMLy-40 groups, but Homa-B or high-density lipoprotein (HDL) did not significantly increase. Additionally, the FBG, HbA1c, Homa-IR, total cholesterol, triglyceride, and low-density lipoprotein levels were not significantly decreased than in the group treated with metformin alone.
CONCLUSION: Lycopene can enhance the ability of metformin to improve the glycemic status, insulin resistance, beta-cell capacity, and lipid profile of T2DM rats.
KEYWORDS: dyslipidemia, Homa-B, insulin resistance, lycopene, metformin, type 2 diabetes mellitus

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Introduction

The two most significant indicators of type 2 diabetes mellitus (T2DM) are insulin resistance and pacreatic beta-cell disfunction.(1) The homeostatic model assessment for insulin resistance (Homa-IR) and homeostasis model assessment of β-cell function (Homa-B) are significant indicators of T2DM.(2) Previous research results showed that these two indicator have a positive correlation with diabetes.(3) The metabolism of fat and glucose disorder, such as lipotoxicity and glucotoxicity, have a significant

impact on pancreatic β-cell dysfunction. It is anticipated that controlling pancreatic β-cells will enhance insulin synthesis, glucose and fat metabolism.(4)

Uncontrolled T2DM might have greater risk for the development of dyslipidemia and a lack of endothelial progenitor cells (EPCs) (5), which can lead to cardiovascular complications. Complications in T2DM persist even when blood glucose is immobilized because of the metabolic memory phenomenon.(6) Controlling blood glucose and complications in T2DM using metformin as standard therapy does not guarantee completely reverse disease progression. Preclinical or clinical development research is still required to identify appropriate therapies through many alternative mechanisms of action.(7) The first-line therapy for T2DM is metformin.(8) Long-term metformin medication can cause side effects such as decreased vitamin B12 absorption, poor hematopoiesis and neuropathy (9), and digestive problems. These side effects often lead people to discontinue medication.(10) Metformin can reduce fasting blood glucose (FBG) (11) and cholesterol levels by 24% (12–14). T2DM with hyperlipidemia are given statin therapy as a first-line treatment for dyslipidemia, but its side effects can interfere with heart function.(15)

Previous research on the combination of hypoglycemic therapy with natural bioactive substances were already conducted, such as metformin with a water extract of *Muntingia carabula* leaves, curcumin, *Stevia rebaudiana* Bertoni leaves, and astaxanthin (16–19); insulin with curcumin (20); and aminoguanidine with curcumin (21). These combination therapies can lower blood glucose levels, hypolipidemic, while increasing insulin sensitivity.

Lycopene of 10–40 mg/kgBW can reduce FBG levels by 31–37.5%, HbA1c levels by 41% (22), total cholesterol (TC) levels by 33%, Homa-IR levels by 50–75%, additionally increasing insulin sensitivity (23), and plasma insulin levels by 10–15% (24,25). Lycopene can protect against cardiovascular damage.(26)

Combining metformin and lycopene can improve glycemic status by lowering FBG levels and improving glucose tolerance (27–29), increasing insulin sensitivity in obese rats (30), and improving symptoms of metabolic memory (27). Studies on the impact of combining metformin with other bioactive compounds have been carried out, however there is currently an inadequate amount of information about the combination of metformin and lycopene in relation to T2DM. Further research on the impact of combination therapy with lycopene and metformin on insulin resistance, pancreatic beta cell function, and lipid profiles in T2DM is necessary. Therefore, this study was conducted to analyze the potential of lycopene to enhance the ability of metformin to improve the glycemic status, insulin resistance, beta cell capacity, and lipid profile of T2DM rats.

Methods

Animals Model Intervention

Thirty six-week old, 160-200 g male Wistar rats, were obtained from the Animals Laboratory of the Center for Food and Nutrition Studies, Universitas Gajah Mada, Yogyakarta, Indonesia. All animals were housed at a standard laboratory conditions on a 12 h light/12 h dark cycle, with free access to food and water *ad libitium*. Male Wistar rats were then randomized into six groups (n=5 per group): control (N), T2DM-untreated (D), T2DM + 250 mg/kgBW metformin (DM), T2DM + 250 mg/kgBW metformin + 10 mg/kgBW lycopene (DMLy-10), T2DM + 250 mg/kgBW metformin + 20 mg/kgBW lycopene (DMLy-20), and T2DM + 250 mg/kgBW metformin + 40 mg/kgBW lycopene (DMLy-40). The doses of metformin and lycopene were selected based on previous studies.(27,31)

To create T2DM animal models, the rats were induced with a combination of high-fat diets consisting of 60% Comfeed PAR-s (Japfa Comfeed Indonesia, Jakarta, Indonesia), 27.8% flour, 2% cholesterol, 0.2% folic acid, and 10% lard oil for two weeks (32,33), followed by the administration of a single dose of 45 mg/kg BW streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) and 110 mg/kg BW nicotinamide (NA) (32–34). The T2DM induction was considered successful if the rats' FBG results were >200 mg/dL.(32)

The metformin administered in the form of 99.60% metformin hydrochloride (PT Phapros, Semarang, Indonesia) were dissolved in 1 mL of coconut oil. Meanwhile, the lycopene was administered in the form of powder (Cat No. CAS 502-65-8; Sigma-Aldrich, St. Louis, MO, USA). The metformin and lycopene treatment were administered once daily through the oral route and lasted for 28 days. The protocol of this study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro (No. 28/EC/H/FK-UNDIP/ IV/2022).

Samples Collection

All rats were sacrificed under ketamine anesthesia 28 days after the previous intervention, following an overnight fast. A glass capillary was used to collect blood samples directly from the retro-orbital flexus. Blood was allowed

to coagulate, then serum was separated by centrifugation at 3,500 rpm for 10 minutes. Samples collected were then used for the assessment of FBG levels, HbA1c, insulin serum, Homa-IR, Homa-B, and lipid profile.

Glycemic Status and Lipid Profile Measurement

The glucose oxidase-peroxidase method was used to measure the serum FBG concentration, with a Dyasis reagent kit (Dyasis, Holzheim, Germany) following manufacturer instructions. Through the help of the enzyme catalyst glucose oxidase (GOD), glucose was oxidized by oxygen to produce hydrogen peroxide (H_2O_2) and gluconic acid. Using a peroxidase (POD) catalyst, hydrogen peroxide would react with 4-aminoantipyrine and phenol to produce quinoneimine and water. Quinoneimine was used a blood glucose indicator that displays the FBG levels.

Fasting serum insulin were measured using FineTest enzyme-linked immunosorbent assay (ELISA) (Fine Biotech, Wuhan, China) according to the manufacturer's instructions. The frozen blood sample was centrifuged, treated with an 11-times enzyme conjugate, and diluted once. After two hours of incubation, the sample was washed, added by tetra methyl blue, and then the incubation continued. Samples were read using an ELISA Reader with a wavelength of 450 nm.

HbA1c were measured using FineTest ELISA (Fine Biotech) according to the manufacturer's instructions. The absorbance of the sample was measured at 450 nm using a microplate reader. The basic principle of that method was the quantitative and relative measurement of antigens or antibodies. The HbA1c level was then determined using the calibration curve.

The lipid profiles, including the serum TC, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were determined by enzymatic methods using FineTest (Fine Biotech). The measurement of cholesterol following enzymatic hydrolysis and oxidation serves as the guiding concept. The colorimetric indicator applied was quinoneimine, which was produced by hydrogen peroxide and peroxidase from 4-Aminoantipyrine and phenol.

Homa-IR and Homa B Calculation

The Homa-IR index was calculated according to the following formula: fasting insulin (μU/L) × FBG (mg/dL)/405. Meanwhile, the Homa-B index was calculated according to the following formula: 360 × fasting insulin (microU/L)/FBG (mg/dL) – 63.(35)

Statistical Analysis

All data were presented as the mean±standard deviation (SD). The statistical significance of differences between the data from pre-test and post-test was determined by Repeated-ANOVA and the Friedman test. The statistical significance of differences between the data from different groups was determined by Kruskal-Wallis test followed by the Mann-Whitney test post hoc test, except for HbA1c. The statistical analyses were performed using SPSS software version 23 (IBM Corporation, Armonk, NY, USA). Values of $p<0.05$ were considered to indicate statistical significance.

Results

Induction of T2DM

The induction of T2DM rats with a high-fat diet mixed with STZ-NA was successful, which was shown by the FBG levels >200 mg/dL, lower serum insulin levels, and a higher homa-IR index in the groups of T2DM-induced rats compared to the N group (Table 1).

Lycopene Reduced FBG, HbA1c, and Fasting Serum Insulin in T2DM Rats

There were significant ($p=0.001$) differences in FBG levels before and after treatment among group (Table 1). T2DM-induced rats that received either a single dose of metformin therapy or a combination of metformin and various concentrations of lycopene had greater decreases in FBG levels than the untreated group. FBG of T2DM-induced rats that treated with combination therapy of metformin and lycopene at doses of 20 and 40 mg/kgBW did not show significant decrease and was higher compared to the metformin therapy alone (Figure 1A).

The delta changes of HbA1c levels (Figure 1B) of the groups that received the combination treatment of lycopene and metformin, either alone or in combination with lycopene, had significantly higher ($p<0.05$) than the untreated T2DM group. The mean differences were 37.31, 39.44, and 44.58 ng/mL, respectively. The delta change of HbA1c levels in the metformin and lycopene combination of dose 20 and 40 mg/kgBW groups were not significantly higher ($p>0.05$) than those in the group receiving metformin alone.

The fasting insulin serum level of the group treated lycopene and metformin, either alone or in combination, was significantly increased ($p<0.05$) than that of the untreated T2DM group (Table 1C). The delta changes of fasting serum insulin levels (Figure 1C) of the groups

Table 1. The effect of lycopene and metformin on glycemic status, insulin resistance, beta cell capacity, and lipid profile of T2DM rats in various groups.

Parameter	Groups						*p*- value
	N	D	DM	DMLy-10	DMLy-20	DMLy-40	
FBG (mg/dL)							
Pre-test	71.10±2.44	268.75±6.53	273.60±10.38	269.56±4.19	268.70±7.49	269.90±7.50	0.068#
Post-test	76.24±1.35	274.25±5.26	112.26±2.31	128.51±3.60	95.63± 2.31	88.81±3.15	
HbA1c (ng/mL)							
Pre-test	23.31±1.40	73.89±1.74	74.51±0.80	73.62±0.58	71.38±0.94	71.22±1.97	<0.001*
Post-test	4.28±0.45	11.18±0.19	5.52±0.08	6.04±0.11	5.02±0.23	4.72±0.08	
Insulin (µIU/mL)							
Pre-test	16.51±1.29	12.81±0.22	12.79±0.27	12.68±0.27	12.62±0.19	12.81±0.19	<0.001*
Post-test	16.26±0.16	12.59±0.19	15.30±0.06	14.28±0.12	15.58±0.16	15.99±0.09	
Homa-IR index							
Pre-test	2.90±0.10	8.50±0.35	8.65±0.47	8.44±0.44	8.37±0.32	8.54±0.30	<0.001*
Post-test	3.06±0.07	8.52±0.30	4.24±0.29	4.53±0.13	3.68±0.11	3.50±0.11	
Homa-B index							
Pre-test	786.13±218.45	22.42±0.34	21.90±0.81	22.12±0.69	22.11±0.67	22.30±0.76	0.068#
Post-test	446.06±49.85	21.46±0.22	112.02±5.46	78.68±4.38	172.59±12.10	225.79±29.19	
TC (mg/dL)							
Pre-test	81.23±2.03	189.31±3.92	186.04±3.18	186.02±3.01	185.48±2.88	187.95±3.38	<0.001*
Post-test	83.07± 2.46	191.11± 3.82	107.13±1.47	120.77±1.92	99.92±1.76	96.71±2.45	
TG (mg/dL)							
Pre-test	70.67±3.08	136.26±2.97	135.27±1.91	128.76±1.83	128.06±2.47	128.20±3.49	<0.001*
Post-test	72.29±3.06	137.99±3.38	113.57±1.66	128.35±3.38	104.90±2.70	99.28±2.31	
LDL (mg/dL)							
Pre-test	24.58±2.23	75.97±1.05	77.22±1.42	76.39±2.19	75.42±2.28	77.5±1.74	0.068#
Post-test	25.85±1.72	77.54±1.95	40.72±1.50	52.42±1.74	38.12±1.39	33.36 ±2.67	
HDL (mg/dL)							
Pre-test	81.22±2.18	26.12±1.24	24.49±1.18	25.58±1.03	23.95±1.95	24.35±1.31	<0.001*
Post-test	79.71±1.92	24.82±1.12	56.31±1.71	43.55±3.67	63.69 ±1.37	71.63±2.51	

Data presented as Mean±SD (n=5 in each group). *Repeated-ANOVA test, significant if $p<0.05$. #Friedman test statistical effect of lycopen and metformin in glycemic status, insulin resistance, beta cell capacity, and lipid profile of T2DM rats. N: normal rats, with no treatment; D: rats with T2DM, but no treatment; DM: rats with T2DM, received 250 mg/kgBW metformin; DMLy-10: rats with T2DM, received 250 mg/kgBW metformin and 10 mg/kgBW lycopene; DMLy-20: rats with T2DM, received 250 mg/kgBW metformin and 20 mg/kgBW lycopene; DMLy-40: rats with T2DM, received 250 mg/kgBW metformin and 40 mg/kgBW lycopene.

receiving metformin and lycopene combination of dose 20 and 40 mg/kgBW groups were significantly higher ($p<0.05$) than those rats in the group that receiving metformin alone, with mean differences of 0.46 and 1.21 µlU/dL, respectively.

Lycopene Combined with Metformin Improves Insulin Sensitivity and Beta Cell Capacity

The effect of lycopene on enhancing the effects of metformin on Homa-IR and Homa-B cells in each group after 28 days of treatment was shown in Table 1. The Homa-IR (Figure 2A) was significantly decreased ($p<0.001$), and the Homa-B (Figure 2B) was significantly increased in the group of rats were treated with metformin alone or in combination with lycopene than in the T2DM untreated groups.

Antihyperlipidemic Effect of The Combination of Lycopene and Metformin

The lipid profile of each group was shown in Figure 3. At the end of the study, the TC, TG, and LDL levels of all intervention groups significantly decreased ($p<0.05$), and HDL levels decreased compared to those of the T2DM group that received no treatement (Figure 3A-D). Furthermore, after 28 days of treatment with metformin and lycopene, the levels of TC, TG, and LDL in the DMLy-20 and DMLy-40 groups were not significantly decreased ($p>0.05$) than those in the group of T2DM rats received metformin alone, and HDL levels were not significantly increased ($p>0.05$). These findings provide compelling evidence that the addition of lycopene could increase the antihyperlipidemic impact of metformin, but not statistically significant.

Discussion

This study demonstrated that lycopene can improve the effectiveness of metformin in terms of improving the glycemic status, insulin resistance, beta cell capability, and lipid profile in T2DM rats. Insulin resistance, also known as diminished insulin sensitivity, is an important feature of T2DM and results in impaired glucose and lipid metabolism. Metformin is the most appropriate therapy for treating T2DM, and it can be continued as long as it is tolerant and there are no contraindications. If first-line treatment does not improve the T2DM condition, a combination of metformin and additional hypoglycemic therapy might be initiated immediately.(8) The use of herbal therapy in T2DM has been extensively investigated.(36,37)

In terms of enhancing glycemic status, lipid profile, insulin sensitivity, and pancreatic beta cell activity, lycopene plus metformin work in concert. The study's findings demonstrated that adding lycopene at doses of 20 and 40 mg/kgBW to rats receiving metformin improved glycemic status by lowering FBG and HbA1c levels when compared to those of rats receiving only a single dose of metformin. Previous research has shown that combining lycopene with metformin can reduce FBG levels and HbA1c.(22,38) Combining lycopene and metformin can help lowering HbA1c levels to reach the WHO target. In this study, giving lycopene at doses of 20 and 40 mg/kgBW to T2DM rats receiving metformin therapy can increase insulin production, higher than metformin treatment alone groups. Furthermore, this combination therapy can increase pancreatic beta cell activity by boosting the Homa-B index (Table 1, Figure 1, Figure 2). The findings of this study are consistent with previous study, which found that combining lycopene obtained from lycopene with metformin improved glycemic control, insulin sensitivity, and pancreatic cell function.(25,28,31)

In T2DM, lipid metabolism disorders cause persistent hyperglycemia and insulin resistance. The abnormalities discovered were an increase in TC, TG, and LDL, while HDL values declined. One of the goals of T2DM treatment is to improve dyslipidemia so that cardiovascular problems do not occur. Blood glucose control is one of the WHO's recommendations for treating lipid diseases.(8)

Metformin therapy has been shown to enhance lipid metabolism, although the outcomes are lower when metformin is combined with lycopene. The addition of lycopene to T2DM rats receiving metformin was not statistically significant improved lipid metabolism, but it did result in higher HDL cholesterol levels and lower TG, TC, and LDL cholesterol levels in the DMLy-20 and DMTLy-40 groups compared to the group receiving metformin alone (Table 1, Figure 3). Lycopene can decrease blood glucose levels and insulin resistance. These findings suggest that lycopene can increase the efficacy of metformin in treating hyperglicemic and dyslipidemia. The results showed that LDL levels reached the WHO guideline target of 100

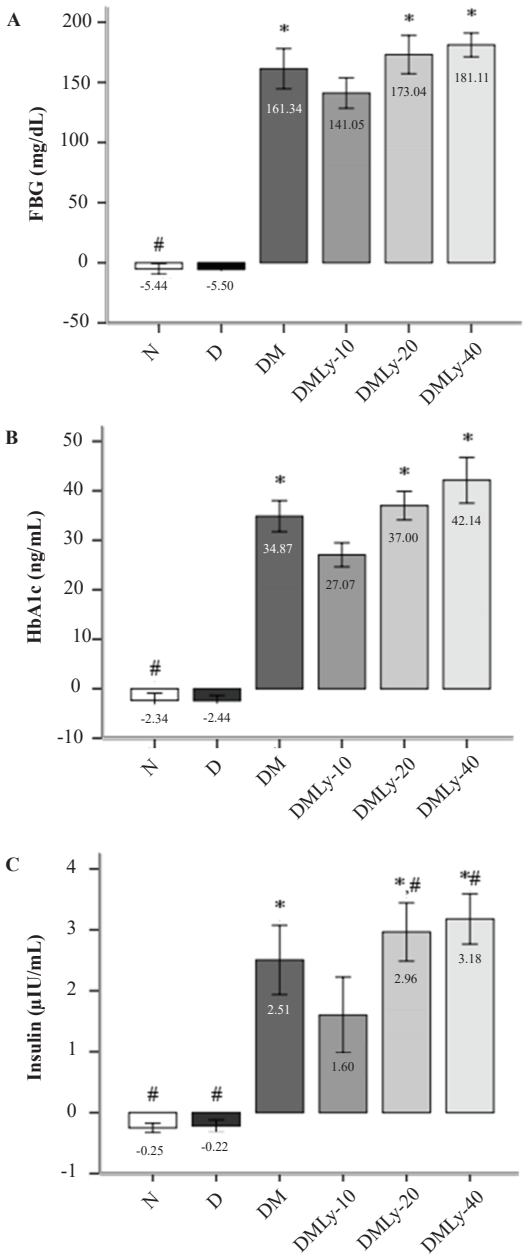

Figure 1. Effect of lycopene and metformin on FBG (A), HbA1c (B), and insulin (C) in rats with T2DM. Values were expressed in terms of the mean delta (post-pre)±SD. Differences in FBG, HbA1c, and insulin levels between groups were analyzed using Kruskal-Wallis test followed by the Mann-Whitney test, *p<0.05 *vs.* D group, #p<0.05 *vs.* DM group.

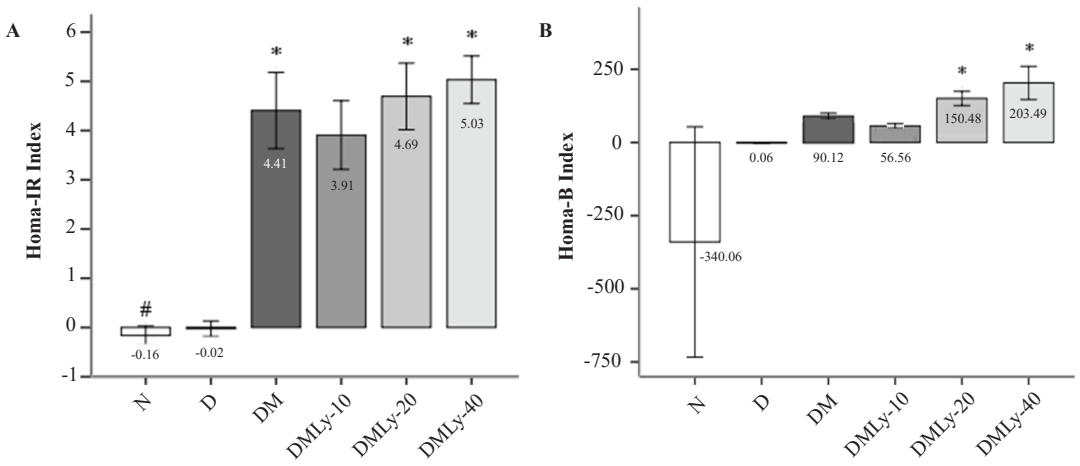

Figure 2. Effect of lycopene and metformin on Homa-IR (A) and Homa-B (B) in rats with T2DM. Values were expressed in terms of the mean delta (post-pre)±SD. Differences Homa-IR, Homa-B levels between groups were analyzed using Kruskal-Wallis followed by the Mann-Whitney test, *p<0.05 *vs.* D group, #p<0.05 *vs.* DM group.

mg/dL (Table 1, Figure 3C). Lycopene has the ability to regulate glycolipid metabolism, prevent insulin resistance, inflammation, and fat accumulation. Additionally, lycopene improves lipid metabolism.(24) It can also increase insulin sensitivity by modulating insulin receptors, insulin-like growth factor-1 (IGF-1) receptors, phosphoinositide 3-kinases (PI3K), and the expression of phosphorylated Akt protein in the cerebral cortex and hippocampus of mice.(39)

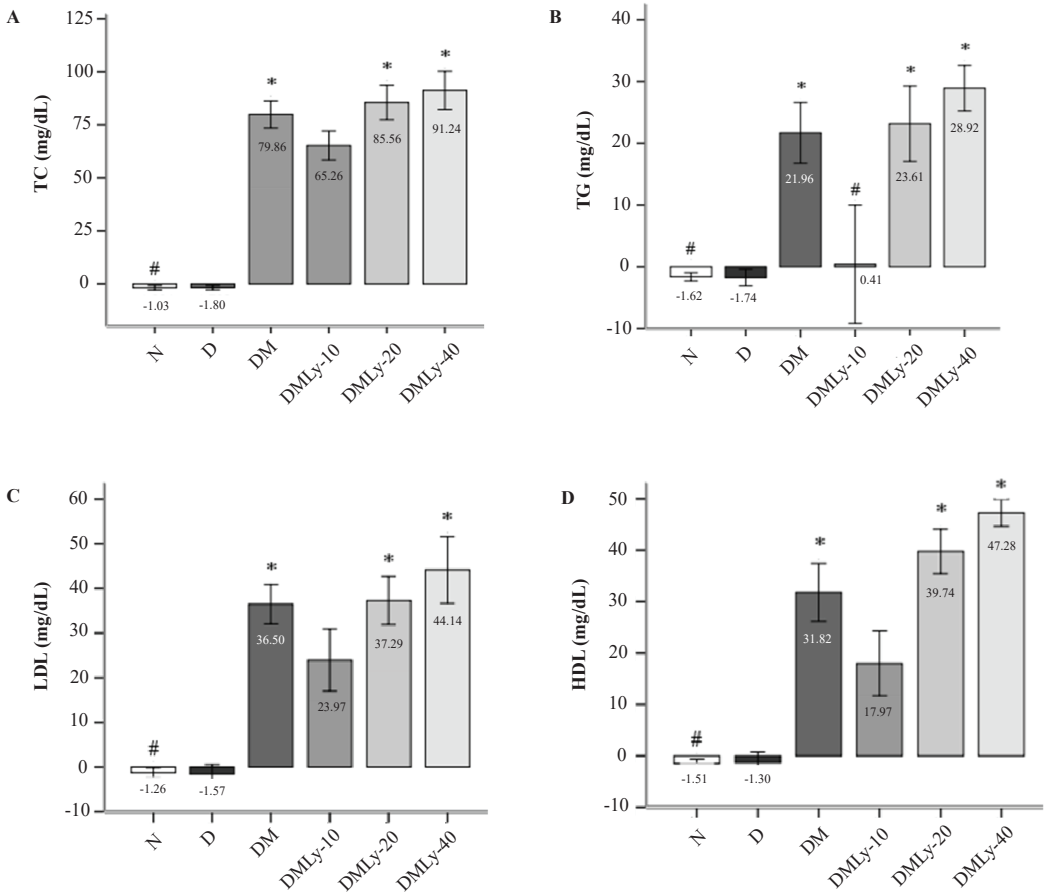

Figure 3. The delta change of TC (A), TG (B), LDL (C), and HDL (D) levels in rats with T2DM. Values were expressed in terms of the mean delta (post-pre)±SD. Differences in TC, TG, LDL, and HDL levels between groups were analyzed using Kruskal-Wallis followed by the Mann-Whitney test, *p<0.05 *vs.* D group, #p<0.05 *vs.* DM group.

Additionally, it can inhibit the liver's expression of signal transducer of activation (STAT3).(40)

Lycopene and metformin together have dose-dependent effects on T2DM rats. The dose that affects the lipid profile, beta cell capacity, insulin resistance, and glycemic status in T2DM rats started at 20 mg/kgBW. This study is limited by the fact that it was conducted only on rats. Therefore, further studies are needed to confirm the effect of the combination of metformin and lycopene in humans.

Conclusion

Based on the results of this study, co-administration of lycopene with metformin to T2DM rats via standard therapy improved the ability of metformin to lower FBG and HbA1c levels and improve insulin sensitivity, pancreatic beta cell function, and lipid metabolism.

Acknowledgments

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Authors Contribution

HS and MS were involved in concepting, methodology, validation, review, investigation, data curation, and editing. HS, MS, and NS were involved in concepting and planning the research, MS performed the data acquisition/collection, MS and DR calculated the experimental data and performed the analysis, MS drafted the manuscript and designed the figures, CD aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

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