RESEARCH ARTICLE

Combination of Metformin and Magnesium Citrate Reduces TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 Expressions in Diabetic Model Rats

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Abstract

BACKGROUND: Diabetes, which causes various complications, involves pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B p65 (NF-κB p65), interleukin-6 (IL-6), cluster of differentiation 4 (CD4), and matrix metalloproteinase-9 (MMP-9). Magnesium has demonstrated anti-diabetic properties, but its anti-inflammatory effects in preventing cardiovascular complications remain unclear. This study aimed to evaluate the anti-inflammatory effects of magnesium citrate, alone and in combination with metformin, by measuring TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 expression in diabetic model rats.

METHODS: Thirty male Wistar rats were divided into five groups: normal control, diabetes control, metformin (treated with 9 mg/200 g/day metformin), magnesium citrate (treated with 3.6 mg/200 g/day magnesium citrate), and combination therapy (treated with 4.5 mg/200 g/day metformin + 1.8 mg/200 g/day magnesium citrate). Diabetes was induced in all groups except the normal control group using streptozotocin (STZ) and nicotinamide (NA). TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 expression levels were measured using enzyme-linked immunosorbent assay (ELISA).

RESULTS: Significant differences in TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 expression levels were observed across all groups ($p<0.001$). The combination therapy group demonstrated the most significant reduction in all parameters compared to the diabetic control group ($p<0.001$) and other therapy groups. Both metformin and magnesium citrate monotherapies showed moderate reductions in cytokine levels but were less effective than combination therapy.

CONCLUSION: Combination therapy with metformin and magnesium citrate exhibited the most potent anti-inflammatory effects, significantly reducing TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 expressions in diabetic Wistar rats. This combination has potential as a therapeutic approach for managing diabetes and its complications.

KEYWORDS: diabetes mellitus, inflammation, cytokines, metformin, magnesium citrate

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Introduction

Diabetes mellitus is a significant public health problem due to its high mortality and morbidity rates.(1–4) In 2021, approximately 529 million people were diagnosed with diabetes, and this number is projected to rise to approximately 1.31 billion by 2025.(1) The International Diabetes Federation predicts a 16% growth in the diabetic population between 2021 and 2045, driven primarily by

aging.(5) The pathophysiological processes of diabetes involve hyperglycemia and insulin resistance, which promote oxidative stress and chronic inflammation by releasing pro-inflammatory cytokines.(6,7)

Hyperglycemia induces molecular changes, including the generation of oxidative stress, leading to increased reactive oxygen species (ROS). Elevated ROS levels trigger the activation of nuclear factor kappa B (NF-κB) p65, a key transcription factor in macrophages. The activation of NF-κB p65 drives the expression of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).(8) In addition to promoting inflammation, hyperglycemia enhances the activity of $CD4^+$ T cells, further amplifying the inflammatory response.(9)

Moreover, increased ROS levels contribute to enzymatic changes, including the upregulation of matrix metalloproteinase-9 (MMP-9) in the aorta. Elevated MMP-9 levels play a significant role in the development of atherosclerosis, a condition closely associated with hyperglycemia.(9) Atherosclerosis is a significant cause of morbidity and mortality worldwide, emphasizing the urgent need for strategies to prevent long-term cardiovascular complications.(10)

Magnesium is an abundant mineral in the human body, serving as a cofactor for enzymes that regulate various processes, including protein synthesis and blood glucose control.(11) It plays a crucial role in insulin secretion and signaling.(12,13) Previous studies show that 11–44% of patients with type 2 diabetes mellitus (T2DM) experience magnesium deficiency.(14–16) This deficiency is associated with a higher risk of cardiovascular events over 10 years (17), leading to the hypothesis that magnesium supplementation may benefit diabetic patients (18).

Magnesium has potent anti-diabetic properties. (12,13) However, its role in preventing cardiovascular complications through its anti-inflammatory effects remains unclear.(17) Similar to metformin, a commonly prescribed drug for diabetes, magnesium has glucose-lowering effects, but its anti-inflammatory properties are not well understood.(19,20) Given that cardiovascular diseases are a leading cause of death in diabetic patients (1–4), further investigation into magnesium's potential protective effects is urgently needed. This study aimed to evaluate the potential anti-inflammatory effects of magnesium citrate in a diabetic rat model by examining levels of TNF-α, NF-κB p65, IL-6, CD4, and MMP-9. Hopefully these findings may contribute to the development of adjunctive treatments for diabetic patients.

Methods

Animal Treatment and Diabetes Induction

Thirty male Wistar white rats received a standard BR1 diet, with the amount adjusted to their average body weight, while they had free access to water (*ad libitum*). Researchers divided the rats into five groups: normal control, diabetes control, metformin, magnesium citrate, and combination therapy. Rats in the diabetes control, metformin, magnesium citrate, and combination therapy groups were intraperitoneally injected with 45 mg/kgBW streptozotocin (STZ) and 110 mg/kgBW nicotinamide (NA) for three consecutive days to induce diabetes. The rats were fasted for 8 hours on the third day to measure fasting blood glucose (FBG) levels to confirm diabetes, defined as FBG ≥200 mg/dL.

After diabetes induction, the diabetes control group received no treatment. The metformin group was treated with 9 mg/200 g/day metformin for 28 days, the magnesium citrate group was treated with 3.6 mg/200 g/day magnesium citrate for 28 days, and the combination therapy group was treated 4.5 mg/200 g/day metformin and 1.8 mg/200 g/day magnesium citrate for 28 days (Figure 1). The experimental study was conducted at Pusat Antar Universitas (PAU), Universitas Gadjah Mada, Yogyakarta. The study protocol was approved by the Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital (No. KE/FK/0244/EC/2024).

Measurement of FBG

The glycemic parameters assessed included fasting blood sugar levels on day 3.(21) FBG levels were measured using a glucose oxidase-peroxidase (GOD-PAP) enzymatic photometric assay (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).(22) Blood samples were collected from the sinus orbitalis and processed to separate serum or plasma. FBG concentrations were determined by measuring the absorbance of a quinoneimine complex at 500 nm. The assay had a 1 mg/dL sensitivity and a measuring range of 1–400 mg/dL. All procedures followed the manufacturer's protocol. Rats with FBG levels ≥200 mg/dL were classified as diabetic.

Measurement of TNF-α, NF-κB p65, IL-6, CD4, and MMP-9

On day 28, the samples, including blood from the sinus orbitalis and abdominal aorta tissues, were collected. Blood

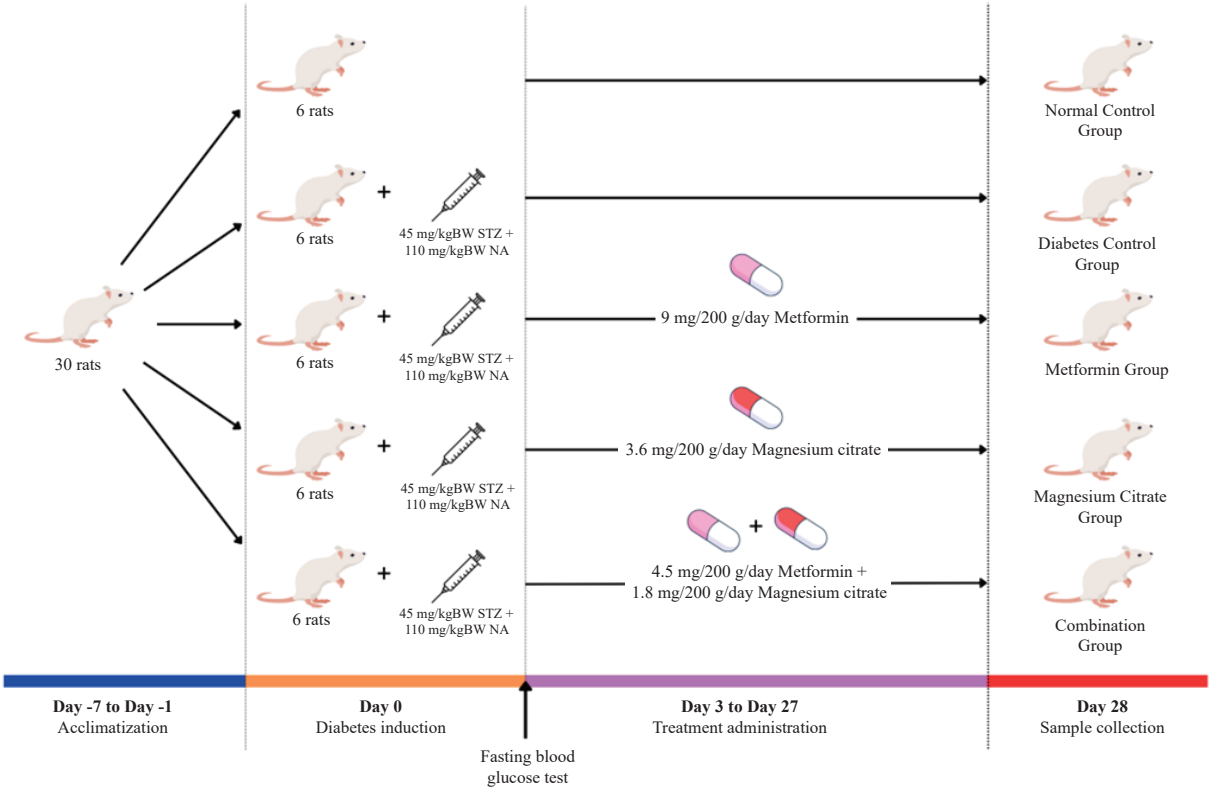

Figure 1. Schematic diagram of study design and treatment timeline in diabetic rats.

samples were used to measure inflammatory markers, including TNF-α, IL-6, and CD4 expressions. Blood samples were allowed to clot for 2 hours at room temperature and then centrifuged at 2000 × g for 20 minutes to separate the serum. The serum aliquots were stored at ≤-20°C until analysis. Meanwhile, abdominal aorta tissue samples were used to analyze NF-κB p65 and MMP-9 expressions.

TNF-α, IL-6, CD4, NF-κB p65, and MMP-9 expression levels were quantified using Rat enzyme-linked immunosorbent assay (ELISA) kits, with following details: Rat TNF-α kit ELISA (Cat. No. ER1393; Fine Test, Wuhan, China) with sensitivity 2.344 pg/mL and detection range 3.906–250 pg/mL; Rat IL-6 kit ELISA (Cat. No. ER0042; Fine Test) with sensitivity 37.5 pg/mL and detection range 62.5–4000 pg/mL; Rat CD4 kit ELISA (Cat. No. ER 0411; Fine Test) with sensitivity 18.75 pg/mL and detection range 31.25–2000 pg/mL; Rat NF-κBP65 kit ELISA (Cat. No. ER 1187; Fine Test) with sensitivity 46.875 pg/mL and detection range 78.125–5000 pg/mL; as well as Rat MMP-9 kit ELISA (Cat. No. ER 0139; Fine Test) sensitivity 46.875 pg/mL and detection range: 78.125–5000 pg/mL. All assays were based on the sandwich ELISA principle and conducted according to the manufacturer's instructions.

Results

Successful Induction of Diabetes with STZ and NA

FBG levels measured on day 3 after diabetes induction were summarized in Table 1. Diabetes, metformin, magnesium citrate, and combination therapy groups all demonstrated FBG levels ≥200 mg/dL, confirming the successful induction of diabetes in these groups. A significant difference in FBG levels was observed among the treatment groups (p<0.05).

Combination Therapy Decreased TNF-α Expression

The TNF-α levels were significantly reduced in all treatment groups compared to the diabetes control group (p<0.05; Table 2, Figure 2A). Combination therapy achieved the lowest TNF-α levels (7.0±0.4 pg/mL) compared to diabetes control (p<0.05) and the other treatment groups. Magnesium citrate and metformin also significantly reduced TNF-α levels compared to diabetes control (p<0.05 for both) but were less effective than combination therapy.

Combination Therapy Reduced NF-κB p65 Expression

NF-κB p65 expression was significantly reduced in all treatment groups compared to the diabetes control group (p<0.05; Table 2, Figure 2B). Combination therapy

Table 1. Fasting blood glucose levels on day 3 after diabetes induction.

Group	FBG (mg/dL)	*p*-value
Normal Control (n=6)	76.4±0.8	0.002*
Diabetes Control (n=6)	269.3±6.2	
Metformin (n=6)	270.4±1.9	
Magnesium Citrate (n=6)	274.0±3.5	
Combination (n=6)	272.6±4.2	

*significant if $p<0.05$, analyzed using Kruskal-Wallis.

resulted in the lowest NF-κB p65 levels (84.2±2.0 ng/mL) compared to diabetes control ($p<0.05$) and the other groups. Furthermore, combination therapy also reduced NF-κB p65 levels compared to metformin ($p<0.05$) and magnesium citrate ($p>0.05$), highlighting its superior effect.

Combination Therapy Suppressed IL-6 Expression

The combination therapy group exhibited the most significant reduction in IL-6 levels (89.7±3.0 pg/mL) compared to the diabetes control group ($p<0.05$; Table 2, Figure 2C). Magnesium citrate and metformin also significantly reduced IL-6 levels compared to the diabetes control group, but the reduction in the combination therapy group was the most pronounced. Additionally, IL-6 levels in the combination therapy group were significantly lower than in the metformin group ($p<0.05$).

Combination Therapy Reduced CD4 Expression

CD4 expression was significantly reduced in all treatment groups compared to the diabetes control group ($p<0.05$; Table 2, Figure 2D). Combination therapy groups achieved the lowest CD4 levels (39.6±0.7 pg/mL) compared to diabetes control ($p<0.05$) and other treatment groups. There were differences observed between combination therapy and metformin ($p<0.05$) as well as magnesium citrate ($p>0.05$).

Combination Therapy Decreased MMP-9 Expression

The MMP-9 levels were significantly reduced in all treatment groups compared to the diabetes control group ($p<0.05$; Table 2, Figure 2E). Combination therapy recorded the lowest MMP-9 levels (9.5±0.2 ng/mL) compared to diabetes control ($p<0.05$) and the other groups. Magnesium citrate (10.4±0.5 ng/mL) also significantly reduced MMP-9 levels compared to the diabetes control group but was not significantly different from combination therapy.

Discussion

Diabetes is a complex metabolic disease that leads to dysfunction in multiple organ systems.(23) Hyperglycemia in diabetes promotes inflammation, oxidative stress, dyslipidemia, and vascular remodeling, vital contributors to disease progression.(7,24) Conversely, hypomagnesemia increases the risk of diabetes (25) and contributes to poorer long-term outcomes (26). Magnesium deficiency exacerbates endothelial dysfunction and inflammation (27) while supplementation has been shown to reduce fasting plasma glucose, glycated hemoglobin, and blood pressure while supplementation has been shown to reduce fasting plasma glucose, glycated hemoglobin, and blood pressure (28) and prevent vascular calcification (29).

TNF-α is a major pro-inflammatory cytokine involved in chronic inflammation and endothelial dysfunction. It induces ROS production in endothelial mitochondria, contributing to atherosclerosis's early stages.(30) TNF-α

Table 2. Effect of metformin, magnesium citrate, and combination therapy on inflammatory marker expression levels in diabetic rats.

Parameter	Group					*p*-value
	Normal Control (n=6)	Diabetes Control (n=6)	Metformin (n=6)	Magnesium Citrate (n=6)	Combination (n=6)	
TNF-α (pg/mL)	5.8±0.2	14.1±0.3	11.5±0.7[a]	9.1±0.4[a]	7.0±0.4[a]	0.000‡
NF-κB p65 (ng/mL)	61.6±5.0	342.7±12.3	97.1±4.6	89.9±3.4[b]	84.2±2.0[bc]	0.000*
IL-6 (pg/mL)	78.0±1.7	140.0±1.5	106.3±1.5	97.3±4.1[b]	89.7±3.0[bc]	0.000*
CD4 (pg/mL)	30.8±0.6	73.8±0.7	49.9±1.0	43.4±1.0[b]	39.6±0.7[bc]	0.000*
MMP-9 (ng/mL)	7.9±0.7	31.5±0.6	13.0±0.4	10.4±0.5[b]	9.5±0.2[bc]	0.000*

‡significant if $p<0.05$, analyzed using one-way ANOVA; *significant if $p<0.05$, analyzed using Kruskal-Wallis; [a]significant with $p<0.05$ (compared to diabetes control group), analyzed using Games-Howell; [b]significant with $p<0.05$ (compared to diabetes control group), analyzed using Bonferroni; [c]significant with $p<0.05$ (compared to metformin group), analyzed using Bonferroni.

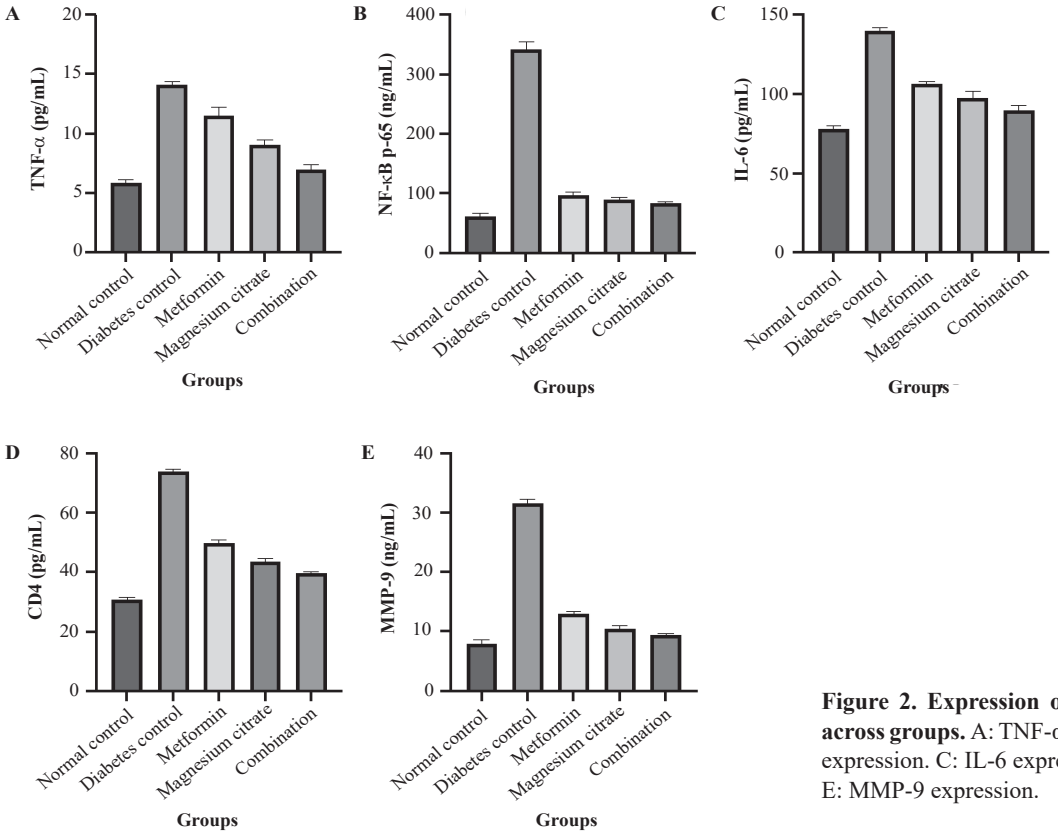

Figure 2. Expression of inflammatory markers across groups. A: TNF-α expression. B: NF-κB p65 expression. C: IL-6 expression. D: CD4 expression. E: MMP-9 expression.

also promotes apoptosis in vascular smooth muscle cells (VSMCs), leading to mineral deposition in atherosclerotic plaques.(31) Our study showed that TNF-α expression was significantly reduced in diabetic rats treated with metformin (11.5±0.7 pg/mL), magnesium citrate (9.1±0.4 pg/mL), or combination therapy (7.0±0.4 pg/mL, $p<0.001$; Table 2), aligning with previous findings.(32) This reduction underscores the anti-inflammatory properties of magnesium citrate and its synergistic effect when combined with metformin, which are critical in mitigating complications associated with T2DM.

NF-κB p65 plays a pivotal role in the initiation of atherogenesis. It facilitates the modification of low-density lipoprotein (LDL) in the vessel wall, triggering local inflammation and the release of chemotactic factors. (8) In our study, NF-κB p65 expression was significantly lower in diabetic rats treated with metformin (97.1±4.6 ng/mL), magnesium citrate (89.9±3.4 ng/mL), or combination therapy (84.2±2.0 ng/mL, $p<0.001$; Table 2). Combination therapy demonstrated superior effects compared to metformin and magnesium citrate, further supporting its potential as a more effective anti-inflammatory treatment for diabetic populations.

IL-6 is a crucial mediator of diabetic complications, including cardiomyopathy and nephropathy. Elevated IL-6 levels contribute to myocardial fibrosis, cardiac hypertrophy, and impaired glucose homeostasis. In hyperglycemic conditions, IL-6 overexpression induces apoptosis and growth arrest.(33) Our study showed that IL-6 expression significantly decreased in diabetic rats treated with metformin (106.3±1.5 pg/mL), magnesium citrate (97.3±4.1 pg/mL), or combination therapy (89.7±3.0 pg/mL, $p<0.001$; Table 2). Notably, combination therapy reduced IL-6 expression significantly more than metformin alone, aligning with prior reviews highlighting magnesium's role in lowering IL-6 levels.(34)

CD4 T cells are involved in the pathogenesis of obesity and insulin resistance. Studies have shown that imbalances in CD4 T cell differentiation are common in patients with T2DM.(35) Additionally, MMP-9 contributes to adipose tissue inflammation and plaque instability by promoting intimal thickening, neovascularization, and the formation of thin fibrous caps.(36) In our study, both CD4 (39.6±0.7 pg/mL) and MMP-9 (9.5±0.2 ng/mL) expression levels were significantly reduced in diabetic rats treated with combination therapy compared to the diabetes control group ($p<0.001$; Table 2). Magnesium citrate and metformin also significantly reduced these markers, but combination therapy consistently yielded the most pronounced effects.

This study demonstrated that the combination therapy of metformin and magnesium citrate had the most potent anti-inflammatory effect in diabetic rats, as evidenced by reduced TNF-α, NF-κB p65, IL-6, CD4, and MMP-9 levels. Metformin, a widely prescribed drug for diabetes, is known to lower glucose levels and suppress inflammation by inhibiting the NF-κB pathway.(19,20) Magnesium, on the other hand, has strong anti-inflammatory properties and enhances the effects of metformin. Together, these two agents produce a synergistic reduction in inflammatory cytokines, making combination therapy more effective than single-agent treatments.

This study is the first to investigate the anti-inflammatory effects of magnesium citrate in diabetic Wistar rats. Previous studies have primarily focused on other treatments, such as palm oil (37), stevia (38), artemisia leaf (39), and others. However, our study has some limitations. First, researchers should validate these findings in higher animal models like primates or humans. Second, the study tested only a single dosage of magnesium citrate; future research could explore dose-dependent effects and determine the safety profile before clinical trials. Additionally, investigating other inflammatory markers could provide a deeper understanding of magnesium's therapeutic potential. Despite these limitations, this study establishes a foundation for the future development of combination therapies to manage diabetes mellitus and its complications.

Conclusion

The combination therapy of metformin and magnesium citrate significantly reduced the expression of TNF-α, NF-κB p65, IL-6, CD4, and MMP-9, demonstrating the most potent anti-inflammatory effects compared to metformin or magnesium citrate alone in diabetic Wistar rats. These findings suggest that combination therapy not only addresses hyperglycemia but also mitigates inflammatory complications associated with diabetes more effectively than single-agent treatments. This combination shows excellent potential as a pharmacotherapy for preventing and/or reducing complications in diabetic patients, warranting further investigation in clinical studies.

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Authors Contribution

RF was involved in research concepting, data acquisition/collection and analysis, result interpretation, figure and/or table design, and manuscript preparation. EP helped analyzing the data. AP aided in interpreting the results. BP, WS, IN, and PD supervised the research. All authors took parts in giving critical revision of the manuscript.

References

1. Ong KL, Stafford LK, McLaughlin SA, Boyko EJ, Vollset SE, Smith AE, *et al.* Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2023; 402(10397): 203–34.
2. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: A review of current evidence. Diabetologia. 2019; 62(1): 3–16.
3. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2017; 14(2): 88–98.
4. Wen P, Luo P, Zhang B, Zhang Y. Mapping knowledge structure and global research trends in gout: A bibliometric analysis from 2001 to 2021. Front Public Health. 2022: 10: 924676. doi: 10.3389/fpubh.2022.924676.
5. Magliano DJ, Boyko EJ; IDF Diabetes Atlas 10th edition scientific committee. IDF Diabetes Atlas [Internet]. 10th Ed. Brussels: International Diabetes Federation; 2021. Chapter 3, Global picture. Available from: https://www.ncbi.nlm.nih.gov/books/NBK581940/.
6. Cagnina A, Chabot O, Davin L, Lempereur M, Maréchal P, Oury C, *et al.* Atherosclerosis, an inflammatory disease. Rev Med Liege. 2022; 77(5–6): 302–9.
7. Li Y, Liu Y, Liu S, Gao M, Wang W, Chen K, *et al.* Diabetic vascular diseases: Molecular mechanisms and therapeutic strategies. Signal Transduct Target Ther. 2023; 8(1): 152. doi: 10.1038/s41392-023-01400-z.
8. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017; 2: 17023. doi: 10.1038/sigtrans.2017.23.
9. Murlistyarini S, Dani AA. Peran matriks metaloproteinase (MMP) pada proses photoaging. J Dermatol, Venereol Aesthet. 2022; 3(1): 13–22.
10. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, *et al.* Heart disease and stroke statistics'2017 update: A report from the American Heart Association. Circulation. 2017; 135(10): e146–603.
11. National Institute of Health [Internet]. Magnesium - Health Professional Fact Sheet [updated 2022 Jun 2; cited 2024 Nov 14]. Available from: https://ods.od.nih.gov/factsheets/Magnesium-

HealthProfessional/.
12. Kostov K. Effects of magnesium deficiency on mechanisms of insulin resistance in type 2 diabetes: Focusing on the processes of insulin secretion and signaling. Int J Mol Sci. 2019; 20(6): 1351. doi: 10.3390/ijms20061351.
13. Gommers LMM, Hoenderop JGJ, Bindels RJM, De Baaij JHF. Hypomagnesemia in type 2 diabetes: A vicious circle? Diabetes. 2016; 65(1): 3–13.
14. Karatas S, Hacıoglu Y, Kose Ş. Magnesium deficiency in type 2 diabetes mellitus and its effect on blood glucose control and diabetes complications. Int J Endocrinol. 2022; 18(2): 104–8.
15. Dasgupta A, Sarma D, Saikia UK. Hypomagnesemia in type 2 diabetes mellitus. Indian J Endocrinol Metab. 2012; 16(6): 1000–3.
16. Azad KM, Sutradhar SR, Khan NA, Haque MF, Sumon SM, Barman TK, *et al.* Serum magnesium in hospital admitted diabetic patients. Mymensingh Med J. 2014; 23(1): 28–34.
17. Yang Z, Zhang Y, Gao J, Yang Q, Qu H, Shi J. Association between dietary magnesium and 10-year risk of a first hard atherosclerotic cardiovascular disease event. Am J Med Sci. 2024; 368(4): 355–60.
18. Agrawal P, Arora S, Singh B, Manamalli A, Dolia PB. Association of macrovascular complications of type 2 diabetes mellitus with serum magnesium levels. Diabetes Metab Syndr. 2011; 5(1): 41–4.
19. Saisho Y. Metformin and inflammation: Its potential beyond glucose-lowering effect. Endocr Metab Immune Disord Drug Targets. 2015; 15(3): 196–205.
20. Karbalaee-Hasani A, Khadive T, Eskandari M, Shahidi S, Mosavi M, Nejadebrahimi Z, *et al.* Effect of metformin on circulating levels of inflammatory markers in patients with type 2 diabetes: A systematic review and meta-analysis of randomized controlled trials. Ann Pharmacother. 2021; 55(9): 1096–109.
21. Pramesthi ADED, Ardana M, Indriyanti N. Drug-herb interaction between metformin and momordica charantia in diabetic mice. Mol Cell Biomed Sci. 2019; 3(2): 81–7.
22. Ahmad MF, Haidar MA, Naseem N, Ahsan H, Siddiqui WA. Hypoglycaemic, hypolipidaemic and antioxidant properties of Celastrus paniculatus seed extract in STZ-induced diabetic rats. Mol Cell Biomed Sci. 2023; 7(1): 10–7.
23. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, *et al.* The role of inflammation in diabetes: Current concepts and future perspectives. Eur Cardiol. 2019; 14(1): 50–9.
24. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian J Endocrinol Metab. 2016; 20(4): 546–51.
25. Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch Biochem Biophys. 2007; 458(1): 40–7.
26. Odusan OO, Familoni OB, Odewabi AO, Idowu AO, Adekolade AS. Patterns and correlates of serum magnesium levels in subsets of type 2 diabetes mellitus patients in nigeria. Indian J Endocrinol Metab. 2017; 21(3): 439–42.
27. Kupetsky-Rincon EA, Uitto J. Magnesium: Novel applications in cardiovascular disease – A review of the literature. Ann Nutr Metab. 2012; 61(2): 102–10.
28. Xu L, Li X, Wang X, Xu M. Effects of magnesium supplementation on improving hyperglycemia, hypercholesterolemia, and hypertension in type 2 diabetes: A pooled analysis of 24 randomized controlled trials. Front Nutr. 2023; 9: 1020327. doi: 10.3389/fnut.2022.1020327.
29. Ter Braake AD, Shanahan CM, De Baaij JHF. Magnesium counteracts vascular calcification: Passive interference or active modulation? Arterioscler Thromb Vasc Biol. 2017; 37(8): 1431–45.
30. Chen X, Andresen1 BT, Hill M, Zhang J, Booth F, Zhang C. Role of reactive oxygen species in tumor necrosis factor-alpha induced endothelial dysfunction. Curr Hypertens Rev. 2008; 4(4): 245–55.
31. Milutinović A, Šuput D, Zorc-Pleskovič R. Pathogenesis of atherosclerosis in the tunica intima, media, and adventitia of coronary arteries: An updated review. Bosn J Basic Med Sci. 2020; 20(1): 21–30.
32. Rostami S. IRCT20210413050957N1: Survey the effect of magnesium citrate supplementation on clinical symptoms and TNF-a and hs-CRP factors in patients with COVID-19. Iranian Registry of Clinical Trials. 2021. Available from: https://irct.behdasht.gov.ir/trial/55660.
33. Zhao L, Hu H, Zhang L, Liu Z, Huang Y, Liu Q, *et al.* Inflammation in diabetes complications: Molecular mechanisms and therapeutic interventions. MedComm. 2024; 5(4): e516. doi: 10.1002/mco2.516.
34. Gröber U, Schmidt J, Kisters K. Magnesium in prevention and therapy. Nutrients. 2015; 7(9): 8199–226.
35. Saigusa R, Winkels H, Ley K. T cell subsets and functions in atherosclerosis. Nat Rev Cardiol. 2020; 17(7): 387–401.
36. Li T, Li X, Feng Y, Dong G, Wang Y, Yang J. The role of matrix metalloproteinase-9 in atherosclerotic plaque instability. Mediators Inflamm. 2020; 2020: 3872367. doi: 10.1155/2020/3872367.
37. Alwahaibi N, Budin SB, Hamid ZA, Mohamed J, Latip J, Ismail NB, *et al.* Tocotrienol rich fraction from palm oil reduces plasma and erythrocyte membrane lipid alteration in diabetic rats: Tocotrienols reduce dyslipidemia in plasma and erythrocytes.Indones Biomed J. 2019; 11(3): 247–56.
38. Lestari K, Ridho A, Nurcayani N, Ramadhania ZM, Barliana MI. Stevia rebaudiana Bertoni leaves extract as a nutraceutical with hypoglycemic activity in diabetic rats. Indones Biomed J. 2019; 11(2): 182–7.
39. Kartikadewi A, Prasetyo A, Budipradigdo L, Nugroho H, Tjahjono K, Lelono A. Artemisia annua leaf extract increases GLUT-4 expression in type 2 diabetes mellitus rat. Indones Biomed J. 2019; 11(1): 78–84.