RESEARCH ARTICLE

Combined Vitamin D and Magnesium Supplementation Improves Insulin, HOMA Indices, Blood Glucose, and Oxidative Stress Markers in Diabetic Rats

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Received date: Nov 20, 2025; Revised date: Dec 31, 2025; Accepted date: Jan 8, 2026

Abstract

BACKGROUND: Diabetes mellitus (DM) is characterized by disturbances in glucose homeostasis, chronic low-grade inflammation, and heightened oxidative stress. Alterations in vitamin D status and magnesium homeostasis are frequently observed in DM and have been implicated in impaired insulin secretion, decreased insulin sensitivity, and dysregulated antioxidant responses. Although both micronutrients have independently demonstrated potential benefits on glycaemic regulation and oxidative balance, the synergistic therapeutic effects of combined vitamin D and magnesium supplementation remain insufficiently elucidated in experimental models of type 2 DM. Therefore, this study was conducted to determine the effects of combined vitamin D and magnesium supplementation on insulin dynamics, glycaemic control, and oxidative stress markers in streptozotocin-induced diabetic rats.

METHODS: Twenty-four male Wistar white rats were divided into 4 groups: normal control, diabetic control, metformin group, and vitamin D + magnesium group. DM was induced using streptozotocin–nicotinamide injection. Glycaemic parameters including insulin, homeostasis model assessment for insulin resistance (HOMA-IR), and homeostatis model assessment of β-cell function (HOMA-β), were evaluated from fasting serum using immunoassay-based analyses; while oxidative stress markers including superoxide dismutase (SOD) and malondialdehyde (MDA) were measured from plasma using colorimetric spectrophotometric methods.

RESULTS: Vitamin D and magnesium combination achieved the greatest reduction in blood glucose. The mean insulin level and HOMA-β index in the vitamin D + magnesium group were significantly higher than in both the diabetic control and metformin groups (p<0.001). In the same group, HOMA-IR and MDA levels were significantly lower, whereas SOD activity was significantly higher compared with diabetic group and metformin group (p<0.001).

CONCLUSION: The combination of vitamin D and magnesium increases insulin and HOMA-β level and decreases HOMA-IR, SOD, and MDA expressions in diabetic Wistar rats.

KEYWORDS: diabetes mellitus, magnesium, vitamin D, insulin resistance, inflammation

Indones Biomed J. 2026; 18(1): 20-7

Introduction

Because of its high mortality and morbidity, diabetes mellitus (DM) remains as a serious public health issue.(1-4) In 2021, 529 million people have been diagnosed with DM, and by 2025, it is estimated that 1.31 billion people will have the disease.(1) Number of people living with DM has been projected by the International Diabetes Federation to contribute to approximately a 16% rise in the global burden of DM.(5) The development and progression of DM are driven by a complex interplay of multiple metabolic, genetic, and environmental risk factors.(6-7). Among these, oxidative stress, which is defined as an imbalance between the generation of reactive oxygen species (ROS) and the capacity of antioxidant defense systems, has emerged as a central mechanism in the pathogenesis of DM. Indices such as superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentrations are widely employed as surrogate markers to quantify oxidative stress status.(8)

Homeostatis model assessment of β-cell function (HOMA-β) reflects pancreatic β-cell function and insulin secretory capacity, which are crucial in the pathophysiology of diabetes mellitus. Vitamin D has been reported to play a role in β-cell function through the presence of vitamin D receptors in pancreatic β-cells and its involvement in calcium homeostasis, which is essential for insulin secretion. Therefore, adequate vitamin D levels may contribute to improved HOMA-β by supporting β-cell viability and insulin synthesis. Vitamin D has also been shown to exert antioxidant effects. Studies have demonstrated that vitamin D supplementation can enhance SOD activity and reduce MDA levels in diabetic models, suggesting its potential role in mitigating oxidative stress associated with diabetes mellitus.(8)

Meanwhile, magnesium is known to be essential for insulin secretion and signaling (9,10), and it has been suggested that magnesium supplementation may be beneficial for patients with diabetes mellitus (11). Magnesium acts as a cofactor for enzymes involved in glucose metabolism and plays a critical role in insulin receptor activity. Furthermore, magnesium supplementation has been observed to improve insulin sensitivity and reduce insulin resistance in diabetic rats, as evidenced by decreased homeostatic model assessment for insulin resistance (HOMA-IR) values and increased insulin levels. (12) By enhancing insulin secretion and improving insulin action, magnesium may also positively influence HOMA-β, reflecting better β-cell function.

Although both micronutrients have independently demonstrated benefits on glycaemic regulation and oxidative balance, the synergistic effects of combined vitamin D and magnesium supplementation on oxidative stress and insulin dynamics remain insufficiently elucidated in experimental models of type 2 DM. Therefore, this study was conducted to evaluate the combined impact of vitamin D and magnesium supplementation on insulin levels, HOMA-β, HOMA-IR, SOD activity, and MDA levels in a diabetic rat model.

Methods

Subjects Grouping and Treatment

This laboratory experimental study was conducted at the Central Inter-University Laboratory (*Pusat Antar Universitas*, PAU), Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Twenty-four male Wistar rats (approximately 2 months old; body weight 170–200 g) were obtained from the institutional animal facility and acclimatized under controlled temperature and light–dark cycles in individual cages, received standard BR-1 chow adjusted to body weight, and had free access to water. After acclimatization, the rats were randomly allocated into four groups (n=6 per group): a normal control group (Group A), a diabetic control group (Group B), a metformin-treated diabetic group (Group C), and a combined vitamin D + magnesium–treated diabetic group (Group D) (Figure 1).

To establish a type 2 DM model, rats in Groups B–D received intraperitoneal injections of 45 mg/kg body weight streptozotocin (STZ) and 110 mg/kg body weight nicotinamide on day-1 to day-3. The diabetic induction was performed, following an established STZ–nicotinamide protocol.(13) Group C received metformin at a dose of 9 mg/200 g/day, administered orally.(13) Meanwhile, Group D was treated with vitamin D at a dose of 0.9 IU/200 g/day and magnesium at a dose of 1.8 mg/200 g/day, both administered orally from day-3 to day-28.(14,15) Figure 2 showed the experimental procedure of the rats, including the STZ- nicotinamide induction, as well as the administration of vitamin D and magnesium. All experimental procedures were approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada – Dr. Sardjito General Hospital (No. 1.433/V/HREC/2024).

Body Weight Measurement

Throughout the study, rats were housed in individual cages at approximately 28°C with a 12-h light/dark cycle, received

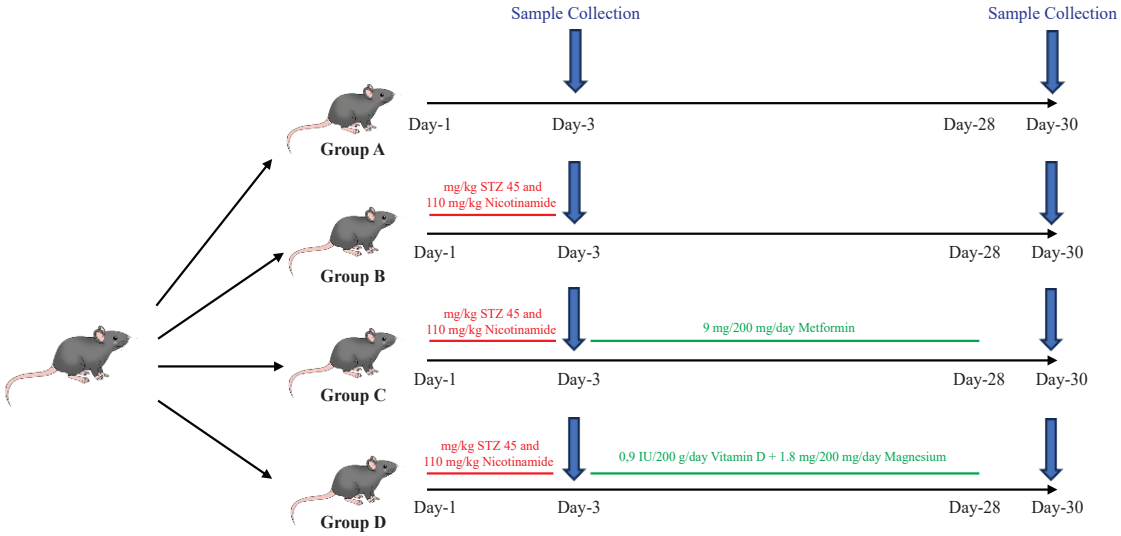

Figure 1. Study scheme of timeline and experimental group allocation.

standard BR-1 chow adjusted to their average body weight, and had free access to water (*ad libitum*). Body weight was recorded at three time points: initial body weight (IBW) on day-0 before diabetes induction, diabetic body weight (DBW) on day-3 after completion of the STZ–nicotinamide injections, and final body weight (FBW) on day-30 at the end of the intervention. At each time point, rats were weighed in the morning after an overnight fast using a digital balance with an accuracy of 0.1 g. Weight gain (WG) and percentage weight gain (%WG) were calculated as WG = FBW − IBW and %WG = [(FBW − IBW) / IBW] × 100, respectively.

Measurement of Insulin, HOMA-IR, and HOMA-β,

Fasting blood glucose was measured from the tail vein on day-10 (pre-treatment) and day-30 (at the end of the intervention) using the Glucose GOD FS kit (Cat. No. 1 2550 99 10 021; DiaSys Diagnostic Systems GmbH, Holzheim, Germany), with a measuring range of 1–400 mg/dL and a detection limit of 1 mg/dL. Fasting blood samples were collected from each animal after an overnight fast (8–12 h). Approximately 3 mL of blood was withdrawn from the retro-orbital sinus into plain collection tubes, allowed to clot at room temperature, and then centrifuged to obtain serum. Serum insulin levels were measured using a Rat INS (Insulin) ELISA Kit (Cat. No. ER1113; FineTest®, Wuhan, China), with a sensitivity of 46.875 pg/mL and a detection range of 78.125–5,000 pg/mL. HOMA indices were calculated from fasting glucose and fasting insulin values.

HOMA-IR was calculated as: HOMA-IR = (fasting insulin [μIU/mL] × fasting glucose [mg/dL]) 405 (or, if glucose was in mmol/L: HOMA-IR = (insulin [μIU/mL] × glucose [mmol/L]) / 22.5). Meanwhile, HOMA-β was calculated as: HOMA-β (%) = (360 × fasting insulin [μIU/mL]) / (fasting glucose [mg/dL] − 63) (or, if glucose was in mmol/L: HOMA-β (%) = (20 × insulin [μIU/mL]) / (glucose [mmol/L] − 3.5)).

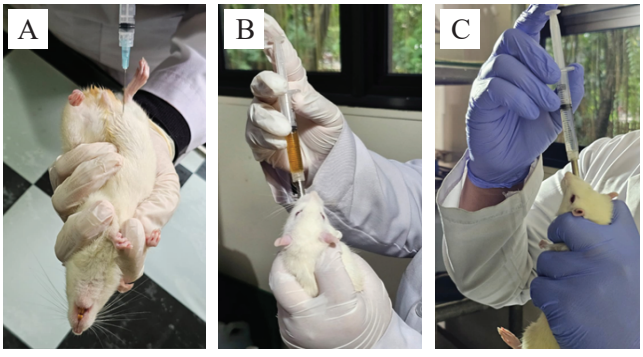

Figure 2. Experimental procedures in the diabetic rat model. A: Intraperitoneal injection technique for STZ–nicotinamide to induce diabetes. B: Oral administration of vitamin D solution using a gastric sonde. C: Oral administration of magnesium solution using a gastric sonde.

Measurement of SOD Activity and MDA Level

For SOD and MDA assessment, 3 mL of blood was collected from the orbital sinus on day-28. The sample was allowed to clot at room temperature for approximately 2 hours and then centrifuged at 2,000 g for 20 minutes. The obtained serum was divided into several aliquots and stored at ≤ –20°C until analysis. SOD activity was measured using a Superoxide Dismutase (SOD) Activity Assay Kit (Cat. No. K335-100; BioVision, Milpitas, CA, USA), based on a colorimetric method at 450 nm, according to

the manufacturer's instructions. MDA concentration was determined with a Mouse MDA (Malondialdehyde) ELISA Kit (Cat. No. ER1878; FineTest), which has a detection range of 7.813–500 ng/mL and a sensitivity of 4.688 ng/mL. All assays were performed according to the manufacturer's instructions, and the results were read using a microplate reader; the data were interpreted by one investigator and independently checked by a biomedical expert.

Statistical Analysis

Statistical analysis was done using IBM® SPSS® Statistics 23 (IBM Corporation, Armonk, NY, USA) for Windows. Data for insulin level, HOMA-β, HOMA-IR, SOD level, and MDA level were presented as mean±standard deviation (SD). Normality test using the Shapiro-Wilk test did not drevealed normal distributions, leading to the utilization of non-parametric Kruskal Wallis test for comparative analysis.

Results

Body Weight Profiles

Body weight profiles for each group were summarized in Table 1. Baseline body weight (IBW) did not differ significantly among Group A to Group D (p=0.456). After diabetes induction, diabetic body weight (DBW) differed significantly among groups (p<0.001), with Group B showing a tendency toward weight loss compared with Group A. At the end of the study (FBW), intergroup differences remained significant (p<0.001). Group A exhibited the greatest absolute body weight gain (+35.2 g; +19.8%), whereas Group B showed a net body weight loss (–25.2 g; –15.2%). In contrast, Group C and Group D demonstrated modest body weight gains of +13.7 g (+7.5%) and +15.8 g (+8.7%), respectively, indicating better preservation of body weight compared with Group B (Table 1).

Combined Vitamin D–Magnesium Markedly Lowers Blood Glucose

Vitamin D and magnesium combination achieved the greatest reduction in blood glucose. Blood glucose was measured at two time points; diabetic blood glucose (DBG; on day-3) and final blood glucose (FBG; on day-30) (Table 2). Baseline (day-0) blood glucose was not recorded; but all rats were obtained from the same healthy colony, maintained under identical conditions, and had not received any intervention before induction, so day-3 values after STZ–nicotinamide injection were used as the reference to confirm successful diabetes induction and to quantify treatment-related changes. On day-3, the normal control group (Group A) had the lowest DBG (76.4±0.8 mg/dL), whereas all induced groups (Group B–D) showed markedly elevated DBG (269.3±6.2, 270.4±1.9, and 268.6±2.6 mg/dL, respectively; p=0.003), confirming successful establishment of the diabetic model.

After 28 days of treatment, FBG remained high in Group B (271.1±6.0 mg/dL), while both Group C and Group D markedly reduced FBG to 123.0±2.5 and 99.4±1.3 mg/dL, respectively (p<0.001 across groups). Relative to day-3 levels, Group C showed a mean decrease of 147.4 mg/dL (–54.5%), whereas the combination group (Group D) exhibited the greatest reduction, 169.2 mg/dL (–63.0%), approaching the normal control group (78.7±1.5 mg/dL), which showed only minimal fluctuation over time (+2.3 mg/dL; +3.0%). These findings indicate that combined vitamin D and magnesium supplementation produced a more pronounced antihyperglycaemic effect than metformin monotherapy.

Combined Vitamin D–Magnesium Improved Insulin Levels, HOMA-β, and Reduced HOMA-IR

Serum insulin levels and HOMA indices were summarized in Table 3. Compared with the normal group (Group A),

Table 1. Body weight of the subjects based on treatment groups.

Group	IBW (g)	DBW (g)	FBW (g)	*p*- value	WG (mg/dL)	% WG
A	178.0±3.4	190.5±3.0	213.2±2.9	0.006*	+35.2	+19.8
B	181.0±6.1	178.3±5.2	155.3±4.0		-25.2	-15.2
C	181.8±4.3	181.1±4.3	195.5±4.4		+13.7	+7.5
D	181.0±2.9	180.1±3.1	196.8±4.1		+15.8	+8.7
***p*- value**	0.456	0.000*	0.000*			

Data are presented as mean±SD (n=6 per group). IBW: initial body weight measured on day-0 before diabetes induction; DBW: diabetic body weight measured on day-3 after completion of STZ–nicotinamide injections; FBW: final body weight measured on day-30 at the end of the intervention after an overnight fast; WG: weight gain (WG = FBW − IBW); %WG: percentage weight gain (FBW-IBW)/IBW×100.

Table 2. Blood glucose levels based on treatment groups.

Group	IBG (ng/mL)	FBG (ng/mL)	*p*- value	ΔBG (mg/dL)	% ΔBG
A	76.4±0.8	78.7±1.5	0.040*	+2.3	+3.0
B	269.3±6.2	271.1±6.0		+1.8	+0.7
C	274.0±3.5	113.3±4.6		-160.7	-58.6
D	268.6±2.7	99.4±1.3		-169.2	-63.0
***p*- value**	0.001*	0.000*			

Data are presented as mean±SD (n=6 per group). IBG: initial (diabetic) blood glucose measured on day-3, after the completion of STZ–nicotinamide induction; FBG: fasting blood glucose measured on day-30 at the end of the intervention after an overnight fast; ΔBG: change in blood glucose (ΔBG = FBG − IBG); %ΔBG: percentage change in blood glucose (FBG-IBG)/IBG×100.

the diabetic control group (Group B) demonstrated lower insulin concentrations and HOMA-β values and higher HOMA-IR, consistent with impaired β-cell function and increased insulin resistance. Both treatment groups (Group C and D) improved these parameters. The group D showed the highest insulin level (535.0±3.8 pg/mL) and HOMA-β (158.8±5.1%), approaching closer to normal values than Group B and Group C. HOMA-IR was also lower in the combination group (Group D) (3.9±0.1 IU/mL) compared with the Group B (8.6±0.2 IU/mL), indicating better insulin sensitivity. Overall, intergroup differences for insulin, HOMA-IR, and HOMA-β were statistically significant (p<0.001 for all) (Table 3).

Vitamin D and Magnesium Restored SOD Activity and Reduced MDA Levels

Oxidative stress markers were also presented in Table 3. The diabetic group (Group B) showed a pronounced reduction in SOD activity (30.6±4.5%) and a marked increase in MDA levels (10.7±0.5 ng/mL) compared with the normal group (Group A), reflecting enhanced oxidative stress. Treatment with metformin partially improved these parameters, whereas the combination of vitamin D and magnesium produced the most favorable profile, with higher SOD activity (74.6±3.1%) and lower MDA levels (3.0±0.1 ng/mL) than Group B. Differences in SOD and MDA between groups were statistically significant (p<0.001 for both) (Table 3).

Discussion

The present study demonstrated that combined vitamin D and magnesium supplementation in a diabetic rat model was associated with improvements in body weight, glycaemic control, insulin dynamics, and oxidative stress markers. The accumulation of adipose tissue is known to exacerbate systemic oxidative stress, which in turn can aggravate disturbances in glucose metabolism and contribute to the development and progression of type 2 DM.(16,17) Emerging evidence also indicates that oxidative stress is not only a downstream consequence of obesity but may actively promote adipogenesis, creating a bidirectional pathological cycle in which excess adiposity and oxidative stress perpetuate each other.(16) Within this context, magnesium plays a crucial role as it is directly involved in cellular energy metabolism through the formation of adenosine triphosphate complexed with magnesium (ATP–Magnesium complex), which represents the biologically active form of intracellular energy.(18) In addition, magnesium is required

Table 3. Levels of insulin, SOD, MDA, HOMA-IR, and HOMA-β based on treatment groups.

Group	Insulin (pg/mL)	SOD (%)	MDA (ng/mL)	HOMA IR (IU/mL)	HOMA ß (%)
A	564.7±3.1	84.4±3.1	1.5±0.1	3.3±0.1	391.8±38.1
B	427.0±3.3	30.6±4.5	10.7±0.5	8.6±0.2	22.2±0.5
C	514.7±4.0	63.1±3.1	3.8±0.3	4.7±0.1	92.8±4.5
D	535.0±3.8	74.6±3.1	3.0±0.1	3.9±0.1	158.8±5.1
***p*-value**	0.000*	0.000*	0.000*	0.000*	0.000*

Data are presented as mean±SD (n=6 per group). Insulin, SOD, MDA, HOMA-IR, and HOMA-β were measured once on day-30 (after 28 days of treatment).

for the activity of key antioxidant enzymes such as SOD which neutralize reactive oxygen species and limit oxidative damage. Higher magnesium intake has been associated with reductions in body mass index and waist circumference, as well as lower blood glucose levels, even among individuals without diabetes.(19) Another study found that administering *Lacticaseibacillus rhamnosus* to a diabetic mouse model improved fasting blood glucose levels, reduced HOMA-IR, and decreased body weight, supporting the role of complementary metabolic strategies in ameliorating insulin resistance and obesity-related dysmetabolism. They act on the same metabolic pathways as vitamin D and magnesium. (20) Consistent with this, a combination of metformin and *Momordica charantia* in a diabetic mouse model produced a more pronounced decrease in blood glucose compared with other treatment groups, further illustrating that rational combination approaches can enhance glycaemic control in experimental diabetes.(21) These observations support the notion that the combination of vitamin D and magnesium may contribute to weight regulation and metabolic improvement in diabetic conditions through attenuation of oxidative stress and optimization of energy metabolism.

Vitamin D has an important role in glucose homeostasis, particularly through its effects on pancreatic β-cell function. The active form of vitamin D, 1,25-dihydroxyvitamin D_3, binds to and activates the vitamin D receptor expressed on β-cells, thereby modulating insulin synthesis and secretion. (22) Beyond glucose metabolism, vitamin D deficiency has also been associated with disturbances in mineral balance, including hypocalcemia in preterm infants, underscoring its broader systemic endocrine role from early life.(23) Deficiency of vitamin D has been linked to impaired β-cell function in both experimental and clinical settings, suggesting its involvement in the conversion of proinsulin into active insulin and in maintaining β-cell integrity.(20) In individuals at increased risk for type 2 DM, vitamin D supplementation has been reported to improve composite indices of insulin sensitivity and insulin secretion, potentially delaying the transition to overt diabetes.(24) However, the magnitude of benefit appears to depend on baseline metabolic status; improvements are more pronounced in subjects with normal or mildly impaired glucose tolerance than in those with advanced β-cell failure.(24) This concept is consistent with the natural history of type 2DM, in which progressive insulin resistance is initially compensated by increased insulin output, followed over time by a decline in β-cell mass of approximately 25–50%, eventually leading to decompensation and chronic hyperglycaemia. Another study further emphasizes the clinical relevance of these mechanisms, showing that in subjects with type 2 DM lower serum nerve growth factor levels were significantly associated with higher HOMA-IR and altered HOMA-β, linking insulin resistance and β-cell dysfunction to chronic diabetic complications such as neuropathy.(25)

Oxidative stress is recognized as one of the central mechanisms in the pathogenesis of type 2 DM, characterized by an imbalance between excessive free radical production and a compromised antioxidant defense system. Increased generation of reactive oxygen species promotes lipid peroxidation, reflected by elevated MDA levels, and disrupts enzymatic antioxidant systems, including diminished SOD activity.(20) In the present study, co-supplementation with vitamin D and magnesium significantly reduced MDA levels and enhanced SOD activity in diabetic rats, indicating a protective effect against oxidative damage induced by chronic hyperglycaemia. These findings are in line with previous clinical data in patients with type 2 DM, in which vitamin D supplementation over several months was associated with reductions in oxidative stress indicators, particularly advanced oxidation protein products, during the early phase of treatment.(25) Although the previous study did not demonstrate a marked decrease in MDA concentrations, the observed changes support the potential role of vitamin D as an antioxidant-modulating agent.(25) Similarly, early-active aerobic exercise in Wistar rats has been shown to increase SOD and gluthatione (GSH) levels and reduce MDA without affecting cognitive performance, indicating that interventions which enhance endogenous antioxidant capacity can effectively attenuate oxidative stress.(26) Consistent with this concept, *Celastrus paniculatus* seed extract in STZ-induced diabetic rats has been reported to lower fasting blood glucose and HbA1c, increase insulin levels, and concomitantly reduce MDA while restoring SOD, catalase, and glutathione-related enzymes, indicating parallel improvements in glycaemic control and antioxidant defenses.(26) Although the active phytochemical constituents and primary pharmacological targets of *Celastrus paniculatus* differ from those of vitamin D and magnesium, both approaches converge on attenuating oxidative stress and preserving β-cell function, which may explain the comparable improvements in glucose homeostasis observed across these experimental models.(26)

Differences in biomarker selection, intervention duration, and baseline oxidative status may account for the heterogeneity of responses across studies. Magnesium may potentiate the antioxidant effects of vitamin D through its function as a cofactor for various antioxidant enzymes,

including SOD, and by stabilizing enzyme structure, attenuating mitochondrial oxidative stress, and protecting pancreatic β-cells from oxidative injury.(20,25) Hence, these mechanisms suggest that combined vitamin D and magnesium supplementation represents a rational strategy to counteract oxidative stress pathways in type 2 DMs.

In addition to the effects on oxidative stress and body weight, the present findings also support an improvement in insulin sensitivity and pancreatic β-cell function with combined vitamin D and magnesium supplementation. Active vitamin D ($1,25(OH)_2D_3$) has been shown to ameliorate multiple metabolic disturbances in type 2 DM models, including reductions in body weight, FBG, and insulin levels, along with improved glucose tolerance. (25) The improvements in insulin, HOMA-β, and HOMA-IR observed in the present study are in line with clinical observations that markers of insulin resistance and β-cell function are strongly related to long-term complications and may therefore represent relevant therapeutic targets.(20,25) By integrating the roles of vitamin D in β-cell regulation and magnesium in insulin signalling and antioxidant defense, the co-supplementation approach evaluated in this study provides a mechanistic explanation for the observed improvements in HOMA indices and oxidative stress markers in the diabetic rat model. These data collectively reinforce the concept that targeting micronutrient deficiencies and oxidative stress may offer an adjunctive avenue to enhance metabolic control in diabetes.

Conclusion

The results of this study demonstrates that combined vitamin D and magnesium supplementation improves metabolic and oxidative profiles in STZ–nicotinamide–induced diabetic rats. Co-supplementation lowered fasting blood glucose, improved insulin sensitivity and β-cell function as reflected by HOMA indices, and attenuated oxidative stress by increasing SOD activity and reducing MDA levels, with effects that were comparable or superior to metformin in several parameters. These findings indicate that correcting vitamin D and magnesium status may offer a potential adjuvant strategy to enhance glycaemic control and limit oxidative damage in type 2 DM.

Acknowledgments

The authors are grateful to Universitas Brawijaya for supporting this research. This study was also funded by RKAT PTNBH Universitas Sebelas Maret Fiscal Year 2023 through the Penelitian Hibah Grup Riset (PENELITIAN HGR-UNS) A scheme under Research Assignment Agreement Number: 228/UN27.22/PT.01.03/2023.

Authors Contribution

BF, YY, HK, and RF were involved in the conception and design of the research. BF, RF, and AF performed data acquisition. BF, AS, and AF conducted data analysis. BF, YY, HK, RF, AS, and AF contributed to the interpretation of the results. BF, YY, HK, RF, AS, and AF were involved in manuscript preparation. BF, AS, and AF designed the figures and/or tables. All authors participated in the critical revision of the manuscript and approved the final version.

Conflict of Interest

The authors declare no conflict of interest.

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