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Bioinorganic Magnesium-based Nanomaterials Using Aqueous Extract of *Monodora myristica* Mitigate Lipid Dysregulation, Atherogenicity, Coronary Risk Factors and Abrogate *β*-Cell Dysfunction via Akt/Nrf2 Antioxidant Enzymes in Streptozotocin-Induced Diabetic Rat

Olakunle Afolabi [1,*], Omotade Oloyede [2], Bukola Aluko [2], Adejoke Obajuluwa [3]

[1] Nanomedicine, Phytomedicine and Toxicology Unit, Biochemistry Programme, Department of Chemical Sciences, Afe-Babalola University. P.M.B 5454, Ado- Ekiti, Ekiti State, Nigeria; afolabiob@abuad.edu.ng (O.A.);
[2] Department of Biochemistry, Ekiti State University, Ado-Ekiti, P.M.B 5363, Ado Ekiti, Ekiti State, Nigeria; ibidun.oloyede@eksu.edu.ng (O.O.); bukola.aluko@eksu.edu.ng (B.A.);
[3] Molecular biology and Toxicology Unit, Biological Science Department, Afe-Babalola University. P.M.B 5454, Ado-Ekiti, Ekiti State, Nigeria; ibitayoao@abuad.edu.ng (A.O.);
* Correspondence: afolabiob@abuad.edu.ng (O.A.).

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Abstract: Nanomedicine has recently played crucial roles in addressing numerous human ailments and complications. Therefore, this study aimed to assess the effect of biogenic magnesium hydroxide nanoparticles from *Monodora myristica* ($Mg(OH)_2$NP-*Mm*) on the antioxidant parameters, and lipid dysfunction-related indices. The mRNA expression of the Akt, Nrf2, and insulin genes in streptozotocin-induced diabetic rats were affected by biogenic magnesium hydroxide nanoparticles from Monodora myristica ($Mg(OH)_2$NP-*Mm*). The $Mg(OH)_2$NP-*Mm* was biosynthesized and characterized by EDX, FTIR, SEM, and UV. In order to induce diabetes, a total of 48 mature male Wistar rats (150-250 g) were randomly separated into 8 groups of six rats and given streptozotocin (55 mg/kg bw). Diabetic animals were treated with 50, 100, 150, and 200 mg/kg bw $Mg(OH)_2$NP-*Mm* for 21 days with $Mg(OH)_2$NP-STD (150 mg/kg bw) and glibenclamide (5 mg/kg bw) as control groups. Characterization of $Mg(OH)_2$NP-*Mm* revealed light-scattering capacity (220-235 nm), size (5-100 nm), shape, probable functional groups, and elemental composition. Results revealed an improved pancreatic antioxidant system, lipid profiles, and a significant ($p < 0.05$) reduction in atherogenic and coronary risk indices. Also, $Mg(OH)_2$NP-*Mm*-treated groups revealed upregulated mRNA expressions of Nrf2, Akt, and insulin genes. It is, therefore, likely that the effects of $Mg(OH)_2$NP-*Mm* could probably be due to its capacity to promote Akt/Nrf2/ARE antioxidant system and improve insulin secretion, both of which are essential for treating DM and related complications.

Keywords: nanomaterials; *Monodora myristica*; Akt/Nrf2/ARE pathway; African nutmeg; diabetes mellitus; streptozotocin; antioxidant system; lipid dysfunction; hyperglycemia.

1. Introduction

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia [1, 2]. Despite advances in awareness, DM remains the leading cause of illness and death worldwide [3]. According to a recent report, DM is the most expensive medical condition by a

wide margin [4]. There are two major types of DM: type 1 (T1DM) and type 2 (T2DM) [5]. However, the pathogenic mechanisms of both types of DM differ significantly [6]. According to studies, T2DM accounts for 90-95% of the diabetic population [7, 8]. There are indications that the prevalence of T2DM varies significantly by geographical region due to environmental and lifestyle risk factors [9]. T1DM and T2DM have a high risk of complications [6, 10]. The cause of this disorder has been linked to the failure of the pancreatic β-cells to appropriately secrete insulin for proper glucose homeostasis, leading to serum hyperglycemia [11].

There is ample evidence that free radicals play a significant role in the onset and progression of diabetes and its complications [12, 13]. Vascular cells, particularly the endothelium, have been identified as the primary source of ROS in hyperglycemia, even at low glucose levels [14]. Consistent exposure of β-cells to proliferated ROS causes pancreatic oxidative stress, which reduces insulin secretion and expression [15]. On the other hand, hyperglycemia has been shown to cause changes in mitochondrial activity, resulting in an increase in the production of reactive oxygen species (ROS) and bioenergetic failure [16]. However, hyperglycemia-induced ROS overproduction has been highlighted to exacerbate insulin resistance by interfering with insulin signals and activating pro-inflammatory signaling pathways [17, 18].

DM has been linked to impaired carbohydrate, lipid, and protein metabolism [19]. It is complicated by a number of other factors that have been linked to the etiology of various human diseases, including lipid dysregulation, cardiovascular diseases (CDs), and so on [20]. Previous research has found a link between lipid metabolism dysfunction (hyperlipidemia), insulin resistance (IR), and pancreatic-cell function [21, 22]. In diabetic-related cardiovascular diseases, hyperlipidemia has been identified as the sole underlying mechanism and independent risk factor [23]. The previous study suggests that lipids homeostasis is important for proper insulin secretory activity of β-cells. The bioaccumulation of lipoproteins in the β-cells has been implicated in the induction of hyperglycemia and beta-cell apoptosis [6]. Also, a dysfunction in lipid metabolism has been reported to interfere with the insulin signaling cascade (i.e., down-regulation of the insulin-resistant substrate (IRS1) and Akt (protein kinase B, a serine/threonine kinase)) and subsequently exacerbate DM by preventing translocation of glucose transporters, thus, causing impairment of glucose homeostasis and thereby rendering the cell resistant to insulin [24]. The Nrf2 (nuclear factor erythroid 2-related factor 2)/ARE (antioxidant response element) pathway has been identified as a target for the prevention, prognosis, and management of T2DM [25]. However, the role of Akt phosphorylation in the translocation of Nrf2 to the nucleus, where it plays an important role in cellular protection against oxidative damage, has been studied [26, 27]. According to reports, Nrf2 activation stimulates the transcription of phase II antioxidant enzyme genes via the ARE pathway [28-30].

Nanotechnology has recently emerged as a rapidly evolving technology that plays an important role in various therapeutic applications [31]. Inorganic compound-mediated nanoparticles, whose structures exhibit significant innovative and enhanced physical, chemical, and biological properties due to their nano-size, have sparked considerable interest over the last few decades [32]. However, nanomaterials' promising therapeutical and biological activities with sizes ranging from 1 to 100 nm have been reported [33-35]. Their sizes, shapes, surface charges, stability, and other physicochemical properties significantly impact their biological activities [36]. Recent research indicates that metal-based NPs are useful in various applications, including antimicrobial agents, drug delivery, drug additives, and food products [37, 38].

Furthermore, there is a new trend in nanoparticle synthesis that uses plant extracts to develop new applications [39]. Plants that are biologically active have been shown to be the best reservoirs of diverse phytochemicals for synthesizing biogenic NPs. Thus, due to their simplicity, inherent safety, and affordability, green and rapid nanoparticle synthesis methods based on plant extracts have piqued the interest of researchers [40].

M. myristica, also known as African nutmeg, is a good source of bioactive phytonutrients that can be used as reducing agents in synthesizing metal-based nanoparticles [35, 41]. The purpose of this study was, therefore, to evaluate the possible antidiabetic effect of biosynthesized magnesium hydroxide nanoparticles made from Monodora myristica ($Mg(OH)_2$NP-Mm) on lipid dysregulation, atherogenicity, coronary risk index (CRI), and mRNA expression of AKT, Nrf2 (nuclear factor erythroid 2-related factor 2), and insulin genes in streptozotocin-induced diabetes in Wistar rats.

2. Materials and Methods

2.1. Chemicals and reagents required.

Chemicals and reagents used, such as thiobarbituric acid (TBA), $MgCl_2.6H_2O$, and streptozotocin, were procured from Sigma-Aldrich, Inc., (Saint Louis, MO, USA), Trizol reagent (Inqaba Biotech, Africa, genomics Company, South Africa), cDNA synthesis kit (Maxima H minus first strand cDNA synthesis kit). Other biochemical assay kits were procured from Randox Laboratory Ltd., Crumlin (Antrim, Northern Ireland, UK). Standard magnesium hydroxide nanoparticles (99% purity, $Mg(OH)_2$NP-STD) (American Elements, Los Angeles, CA, USA).

2.2. Seed sample collection.

Dried seeds of M. myristica were purchased from a single merchant at a popular king's market in Ado-Ekiti, Ekiti State, Nigeria. A senior taxonomist at the Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, identified and authenticated the sample with herbarium voucher number: UHAE.2018016.

2.2.1. Preparation of samples.

Pericapyl layers were removed from dried samples of *M. myristica* seeds, and pericapyl-free parts were ground for 50 sec in an electric blender at maximum speed. The blended sample was then defatted with diethyl ether. The fat-containing diethyl ether layer was removed, and the residue was air-dried and stored at room temperature (25 °C) before use.

2.3. Biological synthesis of magnesium hydroxide nanoparticles from M. myristica seeds.

The $Mg(OH)_2$NP-*Mm* was synthesized from a fat-free blended sample of M. myristica seeds using the method of Awwad and Ahmad [42], as described in our previous study [35]. For 10 minutes, a sample of *M. myristica* seeds (10 g) was boiled in 400 mL of sterile distilled water. The solution was allowed to cool at 25 °C before being filtered and centrifuged at 1500 rpm for 5 min. In a typical reaction, 20 mL of the supernatant was added to 300 mL 4 mM $MgCl_2.6H_2O$ and stirred at 25 ℃ for 5 min to obtain a pale yellow solution. Then, 1 M NaOH was drop-wise added to the solution to adjust the pH to above 12. The formation of white colloidal suspension was considered evidence for the formation of $Mg(OH)_2$NP-*Mm* (Figure

1). The solution was then purified by washing it three times with sterile distilled water and centrifuging it three times for 20 minutes at 1800 x g. The precipitated nanoparticles were washed twice with 99% ethanol to obtain powdery nanoparticles and dried at 80 °C.

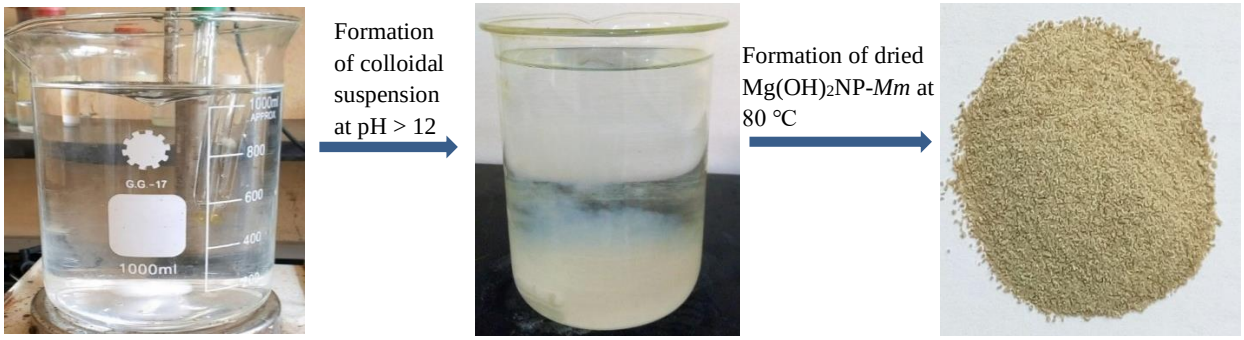

Figure 1. Schematic representation of the biosynthesis of $Mg(OH)_2$NP-*Mm*

2.3.1. Characterization of $Mg(OH)_2$NP-*Mm*

The synthesized $Mg(OH)_2$NP-*Mm* was characterized with a UV-visible scanning spectrophotometer as described by Olajire *et al*. [43]. Also, as stated in our previous study [35], scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), dispersive energy X-ray (EDX), and transmission electron microscopy analyses were performed using the same method of Olajire *et al*.

2.4. Experimental protocols.

2.4.1. Ethical clearance.

The use of experimental animals (rats) involved in this study was carried out strictly with the ethical guidelines for the best practice issued by the ethical clearance committee (ECC) of Afe Babalola University.

2.4.2. Animal treatment.

Forty-eight (48) adult male Wistar rats weighing 150-200 g were obtained from Afe Babalola University's animal house in Ado-Ekiti, Ekiti State, Nigeria. Animals were kept at room temperature (RT) for the duration of the experiment, which lasted 21 days, on a 12-hour light/dark cycle with unlimited access to pelleted animal feed and water. Before the trial began, experimental animals were exposed to ambient conditions for a period of seven days at RT.

2.4.3. Induction of diabetes.

Diabetes was induced according to the method described by Ulas *et al*. [44]. A low dose of streptozotocin (STZ) (55 mg/kg bw) dissolved in cold citrate buffer (0.1 M, pH 4.5, 8°C) was administered via intraperitoneal (IP) into the animals. All experimental rats' fasting blood glucose (FBG) was measured 48 h after diabetes induction; rats with FBG 250 mg/dL were considered diabetic in this study.

2.4.4. Animal grouping.

Experimental animals were divided randomly into 8 treatment groups of 6 rats as follows:

1: Normal control group;
2: Untreated diabetic control group;
3: Diabetic + 50 mg/kg bw $Mg(OH)_2$NP-*Mm*;
4: Diabetic + 100 mg/kg bw $Mg(OH)_2$NP-*Mm*;
5: Diabetic + 150 mg/kg bw $Mg(OH)_2$NP-*Mm*;
6: Diabetic + 200 mg/kg bw $Mg(OH)_2$NP-*Mm*;
7: Diabetic + 150 mg/kg bw $Mg(OH)_2$NP-STD;
8: Diabetic + 5 mg/kg bw glibenclamide.

2.4.5. Collection and preparation of tissues.

2.4.5.1. Preparation of blood sample.

After an overnight fast and the withdrawal of animal feed and water, experimental animals were euthanized with a mild dose of diethyl ether. Blood samples were taken directly from the heart and placed in plain sample bottles. Following that, blood samples were centrifuged at 1800 x g for 10 minutes to obtain the sera used in bioassays.

2.4.5.2. Preparation of tissue homogenate.

Pancreatic tissues were dissected and rinsed in 0.1 M tris buffer (pH 7.4) before being blotted with filter paper and placed on ice. Each sample was weighed before homogenization in 0.1 M tris buffer (1:5 w/v). The homogenate was centrifuged at 3000 rpm for 10 minutes, the pellet was discarded, and the supernatant was stored in the refrigerator for various biochemical assays.

2.4.6. Bioassays.

2.4.6.1. Biochemical analyses.

Antioxidant assays were carried out using the following methods; superoxide dismutase (SOD) [45], catalase (CAT) [46], protein thiol (PT) and non-protein total thiol (NPT) [47], and malondialdehyde produced [48]. Serum lipid profiles were determined as follows; triglyceride level [49], total cholesterol (TC) [50], high-density lipoprotein (HDL) [51], low-density lipoprotein (LDL)-cholesterol and very low-density lipoprotein (VLDL) [52], atherogenic index (AI) [53], CRI [54].

2.4.7. Molecular study.

2.4.7.1. RNA extraction and isolation.

Pancreatic tissues were homogenized in Trizol reagent and stored at approximately 0 °C. The homogenate was treated with a gradient separation medium (chloroform) and centrifuged for 30 minutes at 1500 rpm. For 30 min at 1500 rpm, the supernatant was mixed with 100 mL of precipitating medium (isoamyl alcohol), and the RNA pellet was collected before the supernatant was removed. The precipitated RNA was suspended in nuclease-free water. Samples were digested with DNase and re-precipitated in ethanol to remove any DNA or phenol contamination. UV absorbance spectrophotometry was used to determine total RNA concentration (JENWAY 6305). The RNA was then converted to cDNA using the Proscript ll

second strand synthesis kit. Specific GAPDH gene primers were used to normalize the sample amounts as an internal control.

2.4.7.2. Polymerase chain reaction (PCR) and agarose gel electrophoresis.

Polymerase chain reaction amplification was carried out using On Taq 2x Master Mix (NEB) using the gene-specific primer sets (Table 1). Primers were designed using Primer Express v2.0 (Applied Biosy Team, Foster City, Calif). Amplicons from polymerase chain reactions were separated using 1% agarose gel electrophoresis. UV-gel documentation revealed the relative density by taking snapshots of the DNA bands.

Table 1. Genes and primers used for the mRNA expression.

Genes	Primers
Insulin	Forward: 5′-GTCCTCTGGGAGCCAAG-3′
	Reverse: 5′-ACAGAGCCTCCACCAGG-3′
Nrf2	Forward: 5′-CACATCCAGACAGACACCAGT-3′
	Reverse: 3′-CTACAAATGGGAATGTCTCTGC-5′
AKT	Forward: 5′-TCACCTCTGAGACCGACACC-3′
	Reverse: 5′-ACTGGCTGAGTAGGAGAACTGG-3′
GAPDH	Forward: 5′-AGACAGCCGCATCTTCTTGT-3′
	Reverse: 5′-CTTGCCGTGGGTAGAGTCAT-3′

2.5. Statistical analyses.

One-way ANOVA was used to analyze data, followed by Tukey's test for post-hoc analysis and graphical representation of results using the GraphPad Prism 8.5 program (GraphPad Software, San Diego, CA, USA). All values were given as mean SEM. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

Nanomedicine has recently emerged as a rapidly expanding therapeutic approach to managing and diagnosing various human ailments [55]. In the field of medicine, biologically active nanoparticles with remarkable therapeutic and curative properties have been reported [56]. However, our recent report [35] highlighted the remarkable physicochemical properties (Figure S1) of nontoxic biogenic magnesium-mediated NP in DM. However, these properties, such as shape, functional properties, size, and light scattering power, have been investigated for their potential to confer biological activities on nanoparticles [57].

Figure 2 represents the effects of biosynthesized $Mg(OH)_2$NP-*Mm* on serum lipid profile in STZ-induced diabetic rats. As indicated in the results, administration of synthesized $Mg(OH)_2$NP-*Mm* significantly ($p < 0.05$) improved the level of HDL with a marked significant ($p < 0.05$) reduction in TC, Trig, LDL, and VLDL levels when compared to untreated diabetic control and normal control. Similarly, observation in $Mg(OH)_2$NP-*Mm* treated groups was favorably compared with 150 mg/kg bw $Mg(OH)_2$NP-STD and 5 mg/kg bw glibenclamide-treated diabetic groups. DM has been linked to lipid metabolic dysfunction [58]. According to growing evidence, lipids are also important in the development of diabetic cardiomyopathy and heart failure [59]. Similarly, recent studies have found that changes in plasma and islet lipoprotein levels significantly worsen insulin resistance and islet dysfunction in T2DM [58, 60, 61].

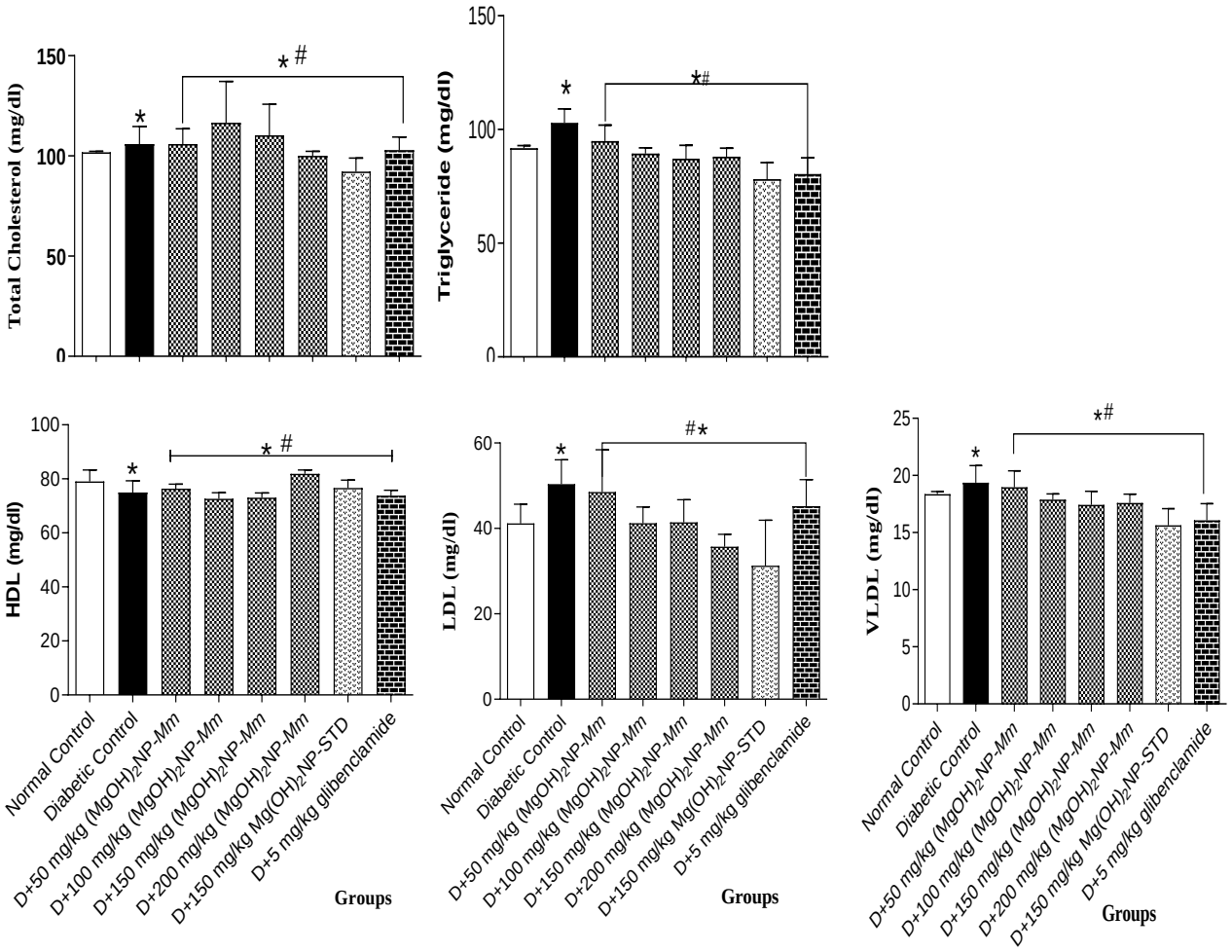

Figure 2. Effects of the biosynthesized $Mg(OH)_2$NP-*Mm* on serum lipid profile in STZ-induced diabetic rats. Results are expressed as mean ± standard error of replicates' mean (SEM). *indicates significant difference at $p < 0.05$ vs normal control; *# ($p < 0.05$) vs diabetic control. Note: D, diabetic.

Figure 3 indicates the effects of the biosynthesized $Mg(OH)_2$NP-*Mm* on atherogenic and CRI. As presented in the results, a significant ($p < 0.05$) decrease was noticeably observed in AI and CRI of $Mg(OH)_2$NP-*Mm* administered groups compared to the untreated diabetic control and normal control. Similarly, this observation was compared favorably with 150 mg/kg bw $Mg(OH)_2$NP-STD and 5 mg/kg bw glibenclamide-treated diabetic groups. As observed in this study (Figure 3), a number of lipid-related indices, such as AI and CRI, are characterized by the high ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) with an increased triglyceride (Trig) and VLDL levels, have been reported as foremost indicators used in the diagnosis of cardiovascular disease (CD), a kind of complication of DM [24]. However, post-administration of $Mg(OH)_2$NP-*Mm* revealed moderated plasma lipoproteins which probably signal the anti-hyperlipidemia effect of the particles that are vital in managing DM and related CDs [24]. This observation's likely mechanism(s) could be linked to the particles' ability to increase insulin secretion and thus inactivate hepatic lipase, reducing -cell lipotoxicity in STZ-induced DM [6, 62 63].

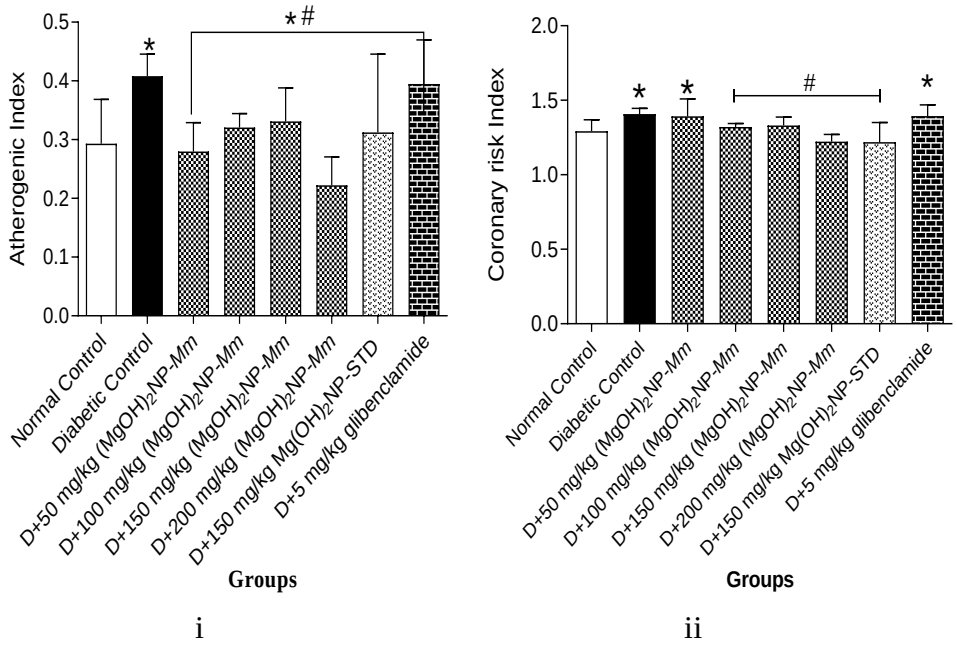

Figure 3. (i-ii): Effects of the synthesized $Mg(OH)_2$NP-*Mm* on the atherogenic index (AI) and coronary risk index (CRI) in STZ-induced diabetic rats. Results are expressed as mean ± standard error of the mean (SEM) of replicates. *indicates significant difference at $p < 0.05$ vs normal control; *# ($p < 0.05$) vs diabetic control. Note: D, diabetic.

Table 2 represents the effects of the biosynthesized $Mg(OH)_2$NP-*Mm* on antioxidant parameters and total protein (TP) of the pancreatic tissue in STZ-induced diabetic rats. In the results, a significant ($p < 0.05$) increase was observed in the levels of protein thiols (PT), non-protein thiols (NPT), catalase (CAT) activity, superoxide dismutase (SOD) activity and total protein (TP) when compared with the untreated diabetic control and a significant ($p > 0.05$) difference when compared with the normal control. Also, there was a significant ($p < 0.05$) decrease in the MDA production of $Mg(OH)_2$NP-*Mm* treated groups compared with the diabetic control and normal control. Similarly, observation in $Mg(OH)_2$NP-*Mm* treated groups was favorably compared with 150 mg/kg bw $Mg(OH)_2$NP-STD and 5 mg/kg bw glibenclamide-treated diabetic groups. Dysfunctional redox homeostasis has long been acknowledged to play a crucial role in the pathogenesis of DM and related complications via various mechanisms [64]. There are indications of an upsurge in the proliferation of ROS in both T1DM & T2DM [65–68]. In this study (Table 2), a decrease in pancreatic endogenous antioxidant systems such as catalase (CAT), superoxide dismutase (SOD), protein thiols (glutathione peroxidase, glutathione reductase, glutathione transferase), and non-protein (reduced glutathione) was observed [69], which could likely reflect STZ-induced pancreatic oxidative stress, which is also evident in malondialdehyde (MDA) levels, a primary biomarker of lipid peroxidation implicated in diabetes. Antioxidant systems have been implicated in reducing and regulating cellular oxidative stress [69, 70].

Nevertheless, following treatment with the synthesized $Mg(OH)_2$NP-*Mm*, a noticeable increase was observed in the cellular-defensive antioxidant components, which suggests a protective effect of the particles on the compromised pancreatic tissue in the STZ-induced hyperglycemic subjects [71]. However, this observation could be credited to various bioactive components such as amine, aromatics, alcohol, ketone, aldehyde, and carboxylic acid functional groups confirmed in $Mg(OH)_2$NP-*Mm* as reported in our previous study [35].

Table 2. Effects of the synthesized $Mg(OH)_2$NP-*Mm* on pancreatic tissue antioxidant status and total protein (TP) in STZ-induced diabetic rats.

Groups	PT	NPT	CAT	SOD	MDA	TP
	(nmol/mg protein)		(nM/min/mg protein)	(U/mg protein)	(µmol)	(mg/ml)
Normal control	4.11 ±1.19	1.51 ±0.78	0.54 ±0.15	15.16 ±1.29	5.56 ±0.46	0.61 ±0.13
Diabetic control	2.88 ±0.22*	0.92 ±0.37*	0.27 ±0.12*	12.67 ±0.49*	6.71 ±0.43*	0.35 ±0.11*
Diabetic + 50 mg/kg $Mg(OH)_2$Ns-*Mm*	4.23 ±0.36**	0.98 ±0.30*	0.17 ±0.07*	13.52 ±2.44**	5.52 ±0.19**	0.45 ±0.04*
Diabetic + 100 mg/kg $Mg(OH)_2$Ns-*Mm*	3.88 ±1.44**	1.01 ±0.26**	0.24 ±0.05*	7.27 ±1.00***	6.31 ±0.22*	0.47 ±0.07*
Diabetic + 150 mg/kg $Mg(OH)_2$Ns-*Mm*	4.50 ±0.35**	1.01 ±0.33**	0.14 ±0.09*	15.87 ±2.00**	6.00 ±0.22*	0.55 ±0.08**
Diabetic + 200 mg/kg $Mg(OH)_2$Ns-*Mm*	3.76 ±1.75**	1.06 ±0.54**	0.07 ±0.02***	20.05 ±0.01***	5.01 ±0.11**	0.82 ±0.42***
Diabetic + 150 mg/kg $Mg(OH)_2$-STD	3.65 ±0.82**	1.03 ±0.13**	0.05±0.02***	10.32 ±0.76*	5.55 ±0.29**	0.70 ±0.10**
Diabetic + 5 mg/kg Glibenclamide	4.69 ±0.28**	1.34 ±0.07**	0.07 ±0.01***	13.92 ±1.97**	5.22 ±0.10**	0.47 ±0.07*

The values in each group are expressed as means ±SEM of replicates (n=6). Different superscripts indicate data comparisons down the column; * indicates a significant difference at $p < 0.05$ vs. normal control; **$p < 0.05$ vs. diabetic control and $p > 0.05$ vs. normal control; and ***p 0.05 vs. diabetic control and $p < 0.05$ vs. normal control.

Figure 4 (i) depicts the relative mRNA expression levels of the Nrf2 gene in STZ-induced diabetic rats after administration of $Mg(OH)_2$NP-*Mm*. The results show a significant ($p < 0.05$) increase in mRNA expression levels of the Nrf2 gene in groups given 50, 100, and 200 mg/kg b.w $Mg(OH)_2$NP-*Mm* for 21 days compared to the untreated diabetic and normal controls. However, there was a significant ($p < 0.05$) decrease in mRNA expression of the Nrf2 gene in the group given 150 mg/kg b.w $Mg(OH)_2$NP-*Mm*. In STZ-induced diabetic groups, this observation was compared favorably to the administration of 150 mg/kg bw Mg(OH)2NP-STD and 5 mg/kg b.w glibenclamide. At the same time, Figure (ii) shows the relative mRNA expression levels of the AKT gene after $Mg(OH)_2$NP-*Mm* administration in STZ-induced diabetic rats. The results indicate a significant ($p < 0.05$) upregulation in the mRNA levels of the AKT gene in groups administered 50, 100, and 200 mg/kg b.w $Mg(OH)_2$NP-*Mm* treated groups when compared with diabetic control and normal control. However, treatment with 150 mg/kg b.w revealed a significant ($p > 0.05$) downregulation compared with diabetic and normal control groups. This observation was compared favorably with the administration of 150 mg/kg bw $Mg(OH)_2$NP-STD and 5 mg/kg b.w glibenclamide in STZ-induced diabetic groups. Akt/Nrf2/ARE pathway has been reported to play a crucial role in cellular defense against oxidative stress [72]. The pathway has been explored as a major mechanism involved in cellular defense for detoxifying and eliminating ROS and other electrophilic agents through conjugative reactions [73, 74].

The observed upregulation in the expression of relative mRNA Nrf2 and Akt genes in this study (Figures 4 i & ii), though not in all the treatment groups, could possibly reflect the ability of the synthesized $Mg(OH)_2$NP-*Mm* to trigger cytoprotection against oxidative damage that characterizes DM [75]. The biosynthesized $Mg(OH)_2$NP-*Mm* could possibly have demonstrated this activity by stimulating the mechanism that activates Akt phosphorylation, thereby prompting nuclear translocation of Nrf2 from the cytoplasm [76, 77], and subsequently initiates the transcription of detoxifying phase II antioxidant proteins such as SOD, CAT, thiol-dependent proteins and non-protein thiol entity [78, 79] as evident in Table 2.

Figure 4(iii) represent the relative mRNA expression levels of the insulin gene following the administration of $Mg(OH)_2$NP-*Mm* in STZ-induced diabetic.

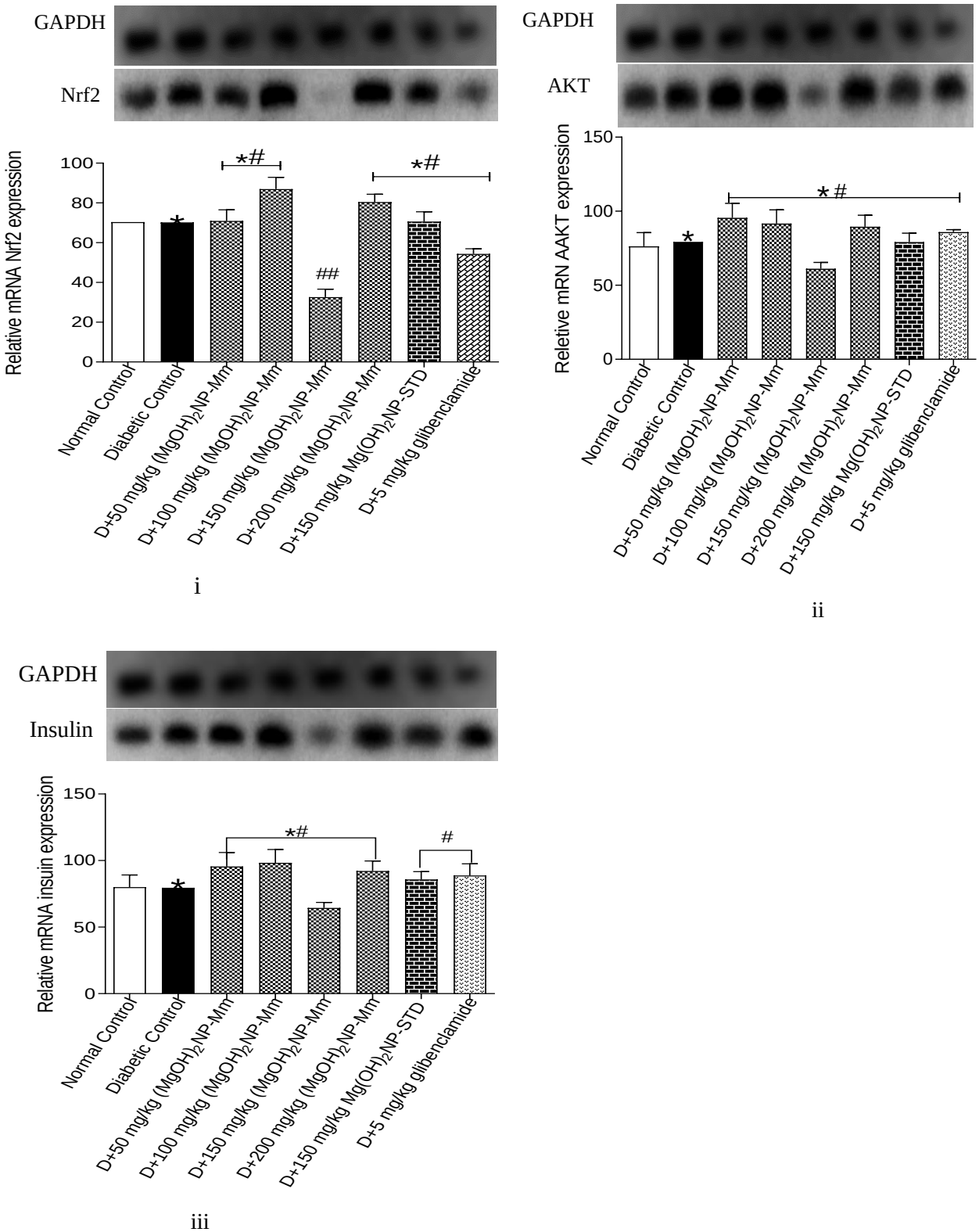

Figure 4. (i-iii): Effects of biosynthesized $Mg(OH)_2$NP-*Mm* on mRNA expressions of Nrf2, AKT and insulin genes in STZ-induced diabetic rats. Results are expressed as mean ± standard error of replicates' mean (SEM). *indicates significant difference at $p < 0.05$ vs normal control; # ($p < 0.05$) vs diabetic control; # ($p < 0.05$) vs normal control; *# ($p < 0.05$) vs normal control and diabetic control; ## ($p < 0.05$) vs diabetic control and other treatment groups. Note: D, diabetic; GAPDH, glyceraldehyde 3-phosphate dehydrogenase (in-house gene).

The results indicate that mRNA levels of insulin gene of groups administered 50, 100, and 200 mg/kg b.w $Mg(OH)_2$NP-*Mm* treated groups were significantly ($p < 0.05$) upregulated compared with diabetic control and normal control. However, 150 mg/kg b.w $Mg(OH)_2$NP-

Mm revealed a significant ($p < 0.05$) decrease in the expression of insulin mRNA level when compared with the untreated diabetic control and normal control. Similarly, this observation was compared favorably with 150 mg/kg bw $Mg(OH)_2$NP-STD and 5 mg/kg b.w glibenclamide-treated diabetic groups. Insulin is a key hormone that plays a central role in the mobilization of glucose into the adipose tissues and the pathogenesis of both forms of diabetes [80, 81]. It interacts with receptors in tissues (skeletal muscle, adipose tissue, and liver) and activates a series of molecular events and biochemical reactions that promote glucose metabolism when serum glucose levels are raised [82, 83]; as such, expression of its mRNA gene in pancreatic *β*-cell is critical in the maintenance of glucose homeostasis [83, 84]. In the study (Figure 4 iii), upregulated mRNA insulin gene was observed relatively among the groups administered the synthesized $Mg(OH)_2$NP-*Mm*, which could substantiate the ability of the particles to moderately trigger insulin secretion. Similarly, the observed reduction in glucose levels following treatment with $Mg(OH)_2$NP-*Mm* (Figure S2) could have been mediated by the ability of the particle to enhance insulin secretion in the pancreatic *β*-cell.

4. Conclusions

This study attempted the synthesis of magnesium hydroxide nanoparticles from *Monodora myristica* seeds ($Mg(OH)_2$NP-*Mm*) via a simple method and appraised its effect in STZ-induced diabetes mellitus (DM) in the rat. However, the biosynthesized $Mg(OH)_2$NP-*Mm* demonstrated ameliorative potential against lipid dysfunction and a marked reduction in atherogenic and coronary risk indices that are vital in managing DM-related cardiovascular diseases. Similarly, the particles improved the pancreatic antioxidant system and significantly upregulated mRNA expression of Nrf2, Akt, and insulin genes. Thus, the antidiabetic effect of Mg(OH)2NP-Mm could probably be credited to its ability to enhance insulin secretion and promote the Akt/Nrf2/ARE antioxidant system that is crucial in managing DM and related complications.

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Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the study described in this manuscript.

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Supplementary materials

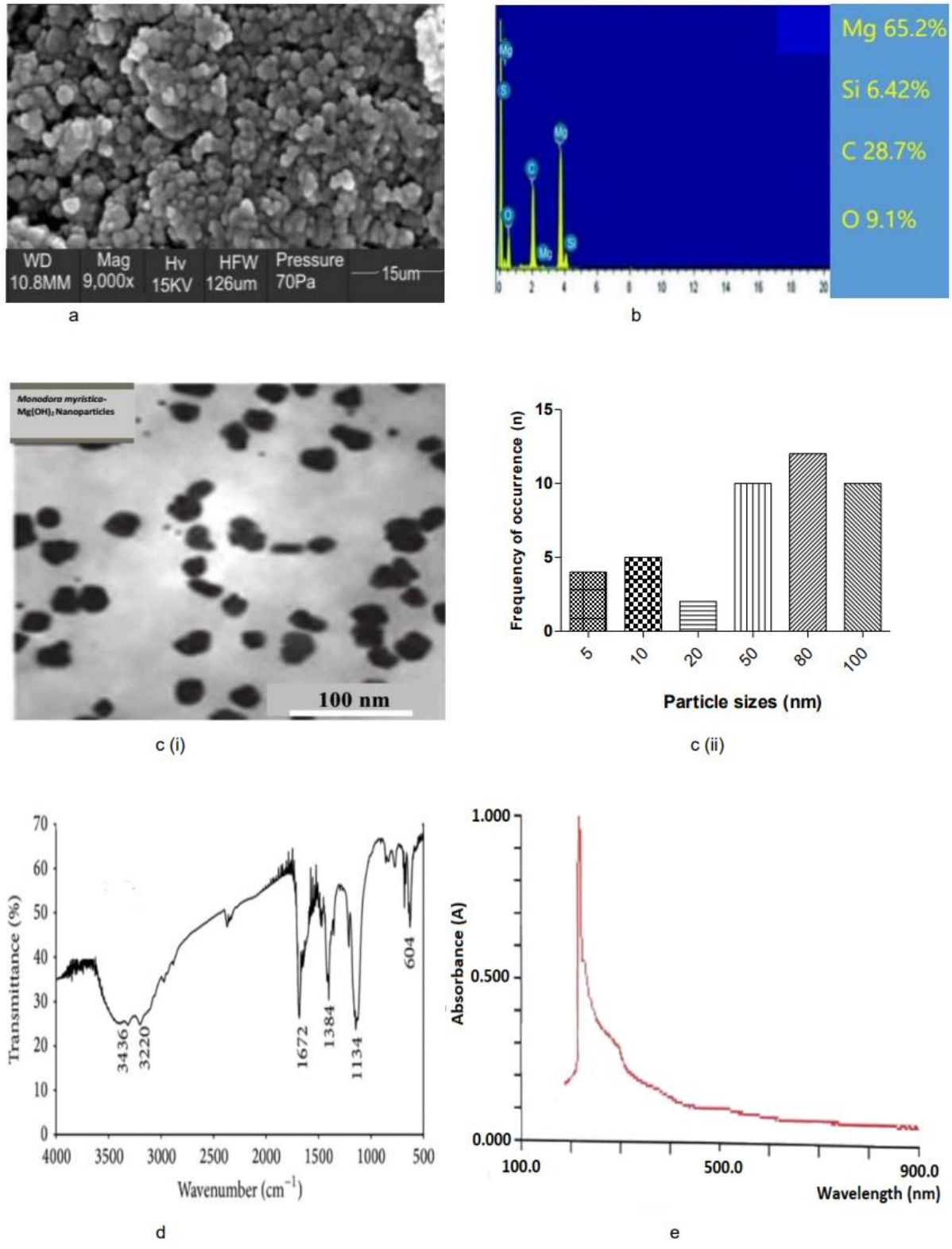

Figure S1. a. SEM (showing morphology of the particles); b. EDX (showing elemental composition of the particles); c (i) TEM image (showing sizes of the particles); c (ii). Frequency of occurrence of the particle sizes; d. FTIR spectra showing functional groups & e. UV-visible spectra showing light absorbing capacity of the synthesized Mg(OH)2NP-Mm.

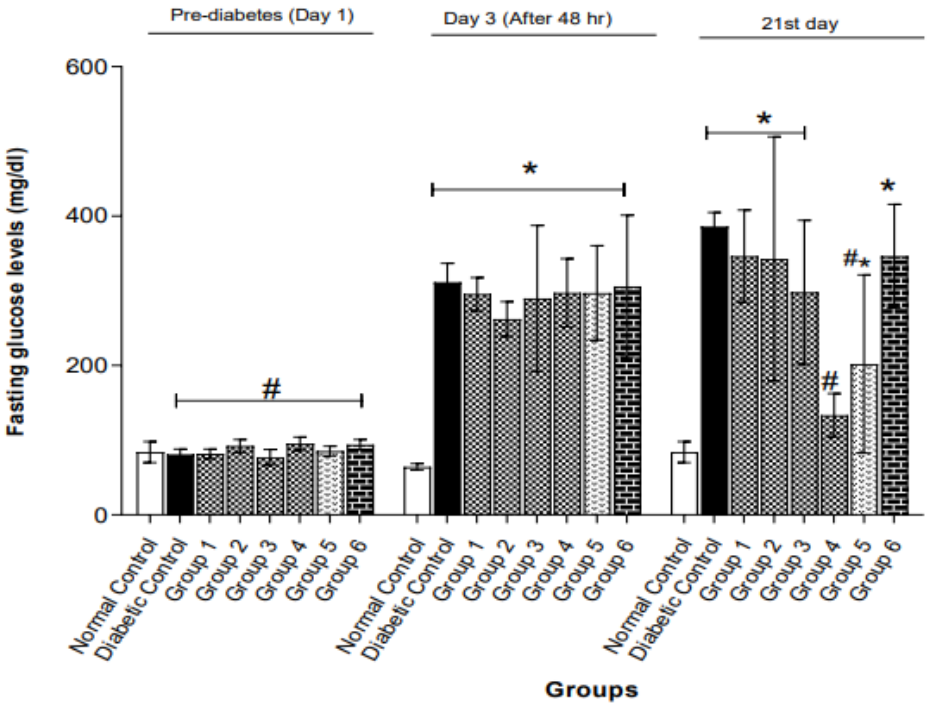

Figure S2. Effects of the biosynthesized Mg(OH)2NP-Mm on fasting blood glucose (FBG) in STZ-induced diabetic rats. Results are expressed as mean ± SEM of replicates. *indicates significant difference at $p < 0.05$ vs normal control; # ($p < 0.05$) vs diabetic control; #* ($p < 0.05$) vs normal and diabetic controls. Keys: Group 1: Diabetic + oral gavage of 50 mg/kg bw Mg(OH)2NP-Mm; Group 2: Diabetic + oral gavage of 100 mg/kg bw Mg(OH)2NP-Mm; Group 3: Diabetic + oral gavage of 150 mg/kg bw Mg(OH)2NP-Mm; Group 4: Diabetic + oral gavage of 200 mg/kg bw Mg(OH)2NP-Mm; Group 5: Diabetic + oral gavage of 150 mg/kg bw Mg(OH)2NP-STD; Group 6: Diabetic + oral gavage of 5 mg/kg bw glibenclamide (Supplementary 3).