Protective effects of phytomediated synthesized magnesium hydroxide nanomaterials using Monodora myristica against diabetes-associated hepatopathy, nephropathy and neuropathy in streptozotocin-exposed rat

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Abstract

There is recently a fast growing interest in green nanotechnology as a better approach for managing diabetes mellitus (DM) and its associated complications. This study therefore aimed at exploring the protective potentials of biogenic phytomediated magnesium-based nanoparticles using Monodora myristica seed (Mg(OH)$_2$NP-Mm) against streptozotocin-induced diabetic hepatopathy, nephropathy and neuropathy in Wistar rats. Mg(OH)$_2$NP-Mm was biosynthesized and characterized using standard techniques. Forty-eight (48) adult male Wistar rats weighing 150-200 g, were indiscriminately grouped into eight (8) groups of six (6) rats (n=6) each. Diabetes was induced with a low dose of streptozotocin (STZ) (55 mg/kg bw) and diabetic animals administered 50, 100, 150 and 200 mg/kg bw Mg(OH)$_2$NP-Mm for 21 days, while control groups received glibenclamide (5 mg/kg bw) and Mg(OH)$_2$-STD (150 mg/kg bw), respectively. In this study, treatment with Mg(OH)$_2$NP-Mm caused a significant (p < 0.05) improvement in fasting blood sugar (FBG), serum hepatic biomarkers (AST, ALT and ALP activities), renal clearance markers (creatinine and urea), total protein (TP) and bilirubin. Enzymatic and non-enzymatic antioxidants, as well as histomorphological examinations indicated a significant (p < 0.05) restoration of the hepatic, renal and brain tissues. This finding therefore, hypothesizes attenuation of redox imbalance which probably could be the basis for the protective effect demonstrated by Mg(OH)$_2$NP-Mm in the tissues examined from STZ-induced diabetic rats.

Introduction

Diabetes mellitus (DM) is a complex endocrine and metabolic dysregulation that poses a high threat on human life (Zhou et al. 2019). It has been acknowledged as one of the highest causes of global mortality (Zheng et al. 2018; Balakumar et al. 2016). A dysregulation in the metabolism of vital biomolecules over a long period has been implicated in DM (Seghrouchni et al. 2002; Kumar et al. 2018). Type I and II are two major categories of DM, subject either to insulin resistance, insufficient insulin secretion or both, resulting into plasma overload of glucose or hyperglycemia (Seghrouchni et al. 2002). Statistics show that type II DM is the most predominant form of DM (ERFC 2010). However, a recent report of world health organization (WHO) indicated a steady increase in the prevalence of both cases over the past few decades (Rodriguez et al. 2002).

DM, when poorly controlled, is frequently associated with severe complications, such as diabetic hepatopathy, nephropathy, neuropathy and cardiomyopathy, etc., (de Oliveira et al. 2015; Pacher et al. 2005). However, vascular complications remain the major cause of morbidity and mortality in the diabetic population (Cooper et al. 2001). The frequent generation of reactive oxygen species (ROS) which perturbs the protective architectures of many tissues has been accentuated to play a vital role in the pathophysiogenesis of DM and its several complications (Johansen et al. 2005; Ceriello 2006; Asmat et al. 2016; Wang et al. 2020; Jiang et al. 2019; Garcia et al. 2001). However, studies have subsequently highlighted some potential sources of ROS in DM to include; auto-oxidation of glucose (Afolabi et al. 2018), modifications in redox balance, declined tissue concentrations of low molecular weight non-enzymatic antioxidants, as well as a compromised enzymatic antioxidant defensive apparatus (Garcia et al. 2001; Haskins et al. 2003). Lipid peroxidation, advanced glycation products (AGEs) of proteins, and DNA damage, etc., has been regarded as good biomarkers of oxidative stress (Piconi et al. 2003; Yamagishi and Matsui 2018).
Recent reports have emphasized the importance of nanomedicine as a potentially better approach for the management DM and its complications (Desai et al. 2021). Synthesis of materials in the range of nanoscale has been described a milestone achievement and new approach in the alternative treatment to many human ailments in the modern-day medicine (Chenthamara et al. 2019; Bhatia 2016). Study shows that nanostructured particles demonstrates more promising ways of improving biologically active products bioavailability, stability, solubility, and distribution (Gera et al. 2017). However, green-synthesis with emphasis on metal-based NP has been reported using plant secondary metabolites (Ali et al. 2016; Manna et al. 2000, Nasrollahzadeh et al. 2019). However, plant extracts are known to be complex mixtures of bioactive molecules such as flavonoids, polyphenols, and phenolic acids (Mittal et al. 2013), that are good sources of capping and reducing agents in the biosynthesis of metal-mediated NP.

*M. myristica*, popularly called African nutmeg, has been reported to be a good source of bioactive compounds such as saponin, alkaloids, cardiac glycosides, steroids, flavonoids, terpenoids and phenols (Afolabi et al. 2021; Gilbert et al. 2011), which could be important sources of reducing agents in the biosynthesis of metal-mediated nanoparticles. Also, magnesium (Mg) is an essential mineral in living system with promising several biological activities (Lin et al. 2004, Makhluf et al. 2005). Oxide and hydroxide of Magnesium NP have successfully been achieved via quite a few methods which include; hydrothermal route, thermal reaction and precipitation method (Taglieri et al. 2015). Magnesium hydroxide NP has been described as nontoxic, noncorrosive, thermally stable with environment-friendly flame retardant (Afolabi et al. 2021). This study therefore was designed to investigate the protective effects of magnesium-based nanomaterials using *M. myristica* seeds against possible complications in STZ-induced diabetes in Wistar rat.

## Materials And Methods

### Chemicals and reagents required

Chemicals and reagents used such as streptozotocin, thiobabituric acid (TBA), MgCl$_2$.6H$_2$O were purchased from Sigma-Aldrich, Inc., (Saint Louis, MO, USA). 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).

### Seed samples collection

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

### Preparation of samples

The pericapyl parts of the dried seeds were removed and pericapyl-free seeds of *M. myristica* were pulverized using an electric blender at the maximum speed for 50 sec. Thereafter, sample was defatted using diethyl ether with a continuous agitation using an automated water-bath shaker for 20 min. Diethyl ether layer with the fat was decanted and the residue was air-dried and stored at room temperature, RT (25 °C) before use.
Biological synthesis of magnesium hydroxide nanoparticles using *M. myristica* seeds

The biosynthesis of Mg(OH)$_2$NP-*Mm* was carried out from the fat-free blended sample of *M. myristica* seeds using the method of Awwad and Ahmad (2004). Freshly dried pericapyl-free sample (10 g) of *M. myristica* seeds was mixed with 400 mL sterile distilled water and boiled for 10 min. The solution was allowed to cool at 25 °C and filtered as previously before centrifuging at 1500 rpm at 25 °C for 5 min to remove the heavy biomaterials. In a typical reaction, 20 mL of the supernatant was added to 300 mL 4 mM MgCl$_2$·6H$_2$O and stirred at 25 °C for 5 min to obtain a pale yellow solution. Then, 1 M NaOH was added drop-wise to the solution to adjust the pH to >12 using a portable pH meter. The white colloidal suspension formed was taken as evidence for the formation of Mg(OH)$_2$NP-*Mm* (Figure 1). The solution was then purified by washing using sterile distilled water and centrifuged 3 times at 1800 x g at 25 °C for 20 min. The precipitated nanoparticles were washed twice using 99% ethanol and then dried at 80 °C in an oven to obtain the materials in powdery form.

Characterization of nanoparticles synthesized from *M. myristica* seeds

Biosynthesized Mg(OH)$_2$NP-*Mm* was characterized using a UV-visible scanning spectrophotometer using the method of Olajire et al. (2016), fourier-transform infrared spectroscopy (FTIR) analysis by the method of John (2000), scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and transmission electron microscopy (TEM) analyses were carried out using the method of Olajire et al. (2016), as detailed in our previous study (Afolabi et al. 2021).

Experimental protocol

Animal treatment

Forty-eight adult male Wistar rats weighing from 150-250 g were obtained from the animal house of Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. The rats were maintained at RT on a 12 h light/dark cycle with free access to pelletized animal feed and water *ad libitum* throughout the experimental period (21 days). Experimental rats were acclimatized with humane condition for 7 days at RT before the commencement of the study. The experiment was carried out with a strict compliance to ethical guidelines issued by the ethical clearance committee (ECC) of Afe Babalola University.

Induction of diabetes

A low dose of streptozotocin (STZ) (55 mg/kg bw) dissolved in cold sodium citrate buffer (0.1 M, pH 4.5, 8°C) was administered intraperitoneally into the animals to induce diabetes using the method of Ulas et al. (2015). Forty-eight hours after the injection of STZ, the fasting blood glucose (FBG) of all the rats were measured. The blood samples used were collected from the tail vein and measured using a portable glucometer (ACCU-CHECK Active). Rats with a FBG ≥ 250 mg/dL were considered diabetic.

Animal grouping

During the 21 days experiments, all rats were fed daily with pelletized animal feed and water *ad libitum*. Experimental animals were divided randomly into 8 groups of 6 rats as follows;

**Group 1**: Normal control group;
Group 2: Untreated diabetic control group;

Group 3: Diabetic group treated + oral gavage of 50 mg/kg bw Mg(OH)$_2$NP-Mm;

Group 4: Diabetic group treated + oral gavage of 100 mg/kg bw Mg(OH)$_2$NP-Mm;

Group 5: Diabetic group + oral gavage of 150 mg/kg bw Mg(OH)$_2$NP-Mm;

Group 6: Diabetic group + oral gavage of 200 mg/kg bw Mg(OH)$_2$NP-Mm;

Group 7: Diabetic group + oral gavage of 150 mg/kg bw Mg(OH)$_2$NP-STD;

Group 8: Diabetic group + oral gavage of 5 mg/kg bw glibenclamide.

Collection and preparation of tissues

Preparation of blood sample

Animals were euthanized by a brief exposure to diethyl ether, after an overnight fasting, following the withdrawal of feed and water. Blood samples were immediately collected by direct heart puncture into both plain sample bottles. Blood samples were subsequently centrifuged at 1800 x g at RT for 10 min to obtain the sera used for various biochemical analyses.

Preparation of tissue homogenates

Tissues such as liver, kidney and brain were subsequently dissected and rinsed in 0.1 M tris-buffer (pH 7.4), blotted with filter paper and placed on ice. Each tissue was weighed and subsequently homogenized in 0.1 M tris-buffer (1:5 w/v). Homogenate was centrifuged at 3000 rpm for 10 min to yield a pellet that was discarded, and the supernatant was kept for various biochemical assays.

Bioassays

Biochemical analyses such as serum FBG was determined using the Accu-chek Advantage II Clinical Glucose meter described by Jenkins and Bakhat (1993), Serum hepatic biomarkers such as serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) by Reitman and Frankel (1957), total protein by Goa (1953). Also, serum creatinine concentration was determined by the method of Spierto et al (1979), urea levels (Fawcett and Scott 1960), total bilirubin (Amadi et al, 2013). Antioxidant enzymes activities of superoxide dismutase (SOD) was studied as described by Misra and Fridovich (1997), catalase (CAT) by Sinha (1972), protein total thiols (PTT) and non-protein total thiol (NPTT) by Ellman (1959), and malondialdehyde (MDA) by the method of Ohkawa et al (1979).

Histological examinations

Histomorphological studies on liver, kidney and brain tissues were carried out according to the method described by Fawcett and Scott (1960).

Data analysis
Data were analyzed using one-way ANOVA, followed by Tukey’s test for post-hoc analysis and graphical representation of results by GraphPad Prism 5 Program (GraphPad Software, San Diego, CA, USA). All values were expressed as mean± SEM, except where otherwise stated. Statistical differences were considered at p < 0.05.

Results

Effect of Mg(OH)$_2$NP-$Mm$ on fasting blood glucose (FBG) in STZ-induced diabetic rat

Table 1 represents FBG observed in STZ-induced diabetic rat following treatment with biosynthesized Mg(OH)$_2$NP-$Mm$. There was a significant (p < 0.05) increase in the FBG of diabetic control compared with normal control. However, treatment with Mg(OH)$_2$NP-$Mm$, triggered a significant (p < 0.05) reduction in FBG, especially in the group administered 200 mg/kg Mg(OH)$_2$NP-$Mm$ compared to diabetic and normal control groups. This observation was favorably compared with Mg(OH)$_2$-STD and glibenclamide treated diabetic groups.

Effect of Mg(OH)$_2$NP-$Mm$ on serum AST, ALT, ALP, bilirubin and TP in STZ-induced diabetic rat

Table 2 represents AST, ALT and ALP activities, bilirubin and TP observed in STZ-induced diabetic rat following treatment with biosynthesized Mg(OH)$_2$NP-$Mm$. As shown in Table 2, there was an evident significant (p < 0.05) increase in serum hepatic-bound ALP, AST, ALT activities and bilirubin in the untreated diabetic control compared to normal control. Also, a significant (p < 0.05) decrease was observed in TP of diabetic control when compared to normal control. However, following the graded administration of biosynthesized Mg(OH)$_2$Ns-$Mm$, a significant (p < 0.05) fall was observed in the activities of these membrane-bound biomarkers compared to the untreated diabetic control. Similarly, a significant (p < 0.05) reduction was observed in bilirubin levels of Mg(OH)$_2$NP-$Mm$ treated groups compared to diabetic control. Similarly, a significant (p < 0.05) increase was observed in TP of Mg(OH)$_2$NP-$Mm$ treated groups compared to the untreated diabetic control and normal control. This observation was favorably compared with Mg(OH)$_2$-STD and glibenclamide treated diabetic groups.

Effect of Mg(OH)$_2$NP-$Mm$ on creatinine and urea in STZ-induced diabetic rat

Table 3 represents creatinine and urea levels observed in STZ-induced diabetic rat following treatment with biosynthesized Mg(OH)$_2$NP-$Mm$. As shown in Table 3, an increase was observed significantly (p < 0.05) in creatinine and urea levels of untreated diabetic control compared to normal control. However, a significant (p < 0.05) difference was noted in creatinine and urea levels of Mg(OH)$_2$NP-$Mm$ treated groups compared to untreated diabetic and normal controls. This observation was compared favorably with Mg(OH)$_2$-STD and glibenclamide treated diabetic groups.

Effect of Mg(OH)$_2$Ns-$Mm$ on enzymatic, non-enzymatic antioxidants, and total protein (TP) of hepatic, renal and brain tissues in STZ-induced diabetic rat

Figure 1, 2 & 3 represent enzymatic and non-enzymatic antioxidants, and TP levels of liver, kidney and brain tissues observed in STZ-induced diabetic rat following treatment with biosynthesized Mg(OH)$_2$NP-$Mm$. A significant (p < 0.05) decrease in PTT, NPTT, CAT, SOD, and TP with an increase (p < 0.05) significantly in MDA
of the diabetic control compared with the normal control. However, following treatment with Mg(OH)$_2$Ns-$Mm$, a significant ($p < 0.05$) increase was observed in PT, NPT, CAT, SOD, and TP with a decrease ($p < 0.05$) significantly in MDA when compared with diabetic control, especially in 200 mg/kg bw Mg(OH)$_2$Ns-$Mm$. This observation was favorably compared with Mg(OH)$_2$-STD and glibenclamide treated diabetic groups.

**Effect of Mg(OH)$_2$NP-Mm on morphological status of hepatic, renal and brain tissues**

Figure 4 (1-8) represents the effect of Mg(OH)$_2$Ns-$Mm$ on morphological status of hepatic tissue in STZ-induced diabetic rats. The untreated diabetic control (Figure 4-1) revealed a shrunk portal and evident necrosis compared to normal control (Figure 4-2) with normal architecture of hepatocytes and portal triad. Treatment with the lowest dose, 50 mg/kg bw Mg(OH)$_2$Ns-$Mm$ (Figure 4-3) revealed a shrunk portal area and mild necrosis compared to untreated diabetic control. However, treatment with 100 mg/kg Mg(OH)$_2$Ns-$Mm$ (Figure 4-4) indicated a normal architecture of hepatocytes and more pronounced portal area compared to untreated diabetic control. Also, treatment with 150 mg/kg Mg(OH)$_2$Ns-$Mm$ (Figure 4-5) shows a mild disruption of hepatocytes whereas, 200 mg/kg Mg(OH)$_2$Ns-$Mm$ (Figure 4-6) shows a shrunk portal area compared to untreated diabetic group. Comparison following treatment with 150 mg/kg Mg(OH)$_2$-STD (Figure 4-7), and 5 mg/kg glibenclamide (Figure 4-8) show normal hepatocyte with poor lobular organizations.

Figure 5 (1-8) represents the effect of Mg(OH)$_2$Ns-$Mm$ on morphological status of renal tissue in STZ-induced diabetic rats. The untreated diabetic control (Figure 5-1) revealed expansion and disruption of glomerulus compared to normal control (Figure 5-2) with normal Bowman’s capsule and urinary space, proximal convoluted tubules and distal convoluted tubules. However, treatment with 50, 100 and 150 mg/kg bw Mg(OH)$_2$Ns-$Mm$ (Figure 5(3-5)) revealed a recovery of glomeruli and convoluted tubules compared to untreated diabetic control. Treatment with 200 mg/kg Mg(OH)$_2$Ns-$Mm$ (Figure 5-6) showed absence of urinary space compared to untreated diabetic group. Comparison following treatment with 150 mg/kg Mg(OH)$_2$-STD (Figure 5-7), and 5 mg/kg glibenclamide (Figure 5-8) revealed normal glomerulus and convoluted tubules.

Figure 6 (1-8) represents the effect of Mg(OH)$_2$Ns-$Mm$ on morphological status of brain tissue in STZ-induced diabetic rats. The untreated diabetic control (Figure 6-1) revealed pyknotic and scantily distributed neuronal cells compared to normal control (Figure 6-2) with evenly distributed normal neuronal cells. However, treatment with 50 and 100 mg/kg bw Mg(OH)$_2$Ns-$Mm$ (Figure 6(3&4)) revealed scantily distributed and mild disrupted neuronal cells compared to untreated diabetic control. Treatment with 150 and 200 mg/kg Mg(OH)$_2$Ns-$Mm$ (Figure 6(5&6)) showed the presence of normal neuronal cell body compared to untreated diabetic group. Comparison following treatment with 150 mg/kg Mg(OH)$_2$-STD (Figure 6-7), and 5 mg/kg glibenclamide (Figure 6-8) revealed recovery of neuronal cells.

**Discussion**

Natural plants are rich sources of reducing agents and have recently been exploited for the biosynthesis of NP (Chu et al. 2002; Sbrana et al. 2014; Omojate et al. 2014). Afolabi et al (2021), recently reported the synthesis of magnesium hydroxide nanomaterials using monodora myristica (Mg(OH)$_2$NP-$Mm$) with various beneficial activities. In the report, characterization of Mg(OH)$_2$NP-$Mm$ revealed morphological shapes, sizes of the
particles and their various functional properties (Afolabi et al. 2021). The characteristic bioactivities of these functional groups, have also been reported (Tu et al. 2012; Oehlke et al. 2014).

In this study (Table 1), increased pre-treatment fasting blood glucose (FBG) observed in the various groups following STZ induction could indicate a sort of derangement in glucose homeostasis (Masiello 2006; Punithavathi et al. 2008). STZ, is a known a drug of choice in islet cell carcinoma and malignant carcinoid tumors that generally shares a related fate of disposition with other nitrosoureas (Zafar and Naqvi 2010). Its diabetogenic, hepatotoxic and nephrotoxic activities have been reported (Piyachaturawat et al. 1991). However, a fall observed in the FBG levels in group administered highest dose of the biosynthesized Mg(OH)$_2$NP-$Mm$ could possibly suggest ability of the nanomaterials to initiate a repair in insulin secretion by the $\beta$-cell, its actions or both (Kalwat and Cobb 2017).

Liver plays an essential role in the regulation of glucose metabolism (Gopalsamy et al. 2011). Elevation of serum hepatic-bound enzymes transaminases (AST & ALT) and phosphatase (ALP), due to their leakage from the hepatic cytosol into the blood stream (Navarro et al. 1993; El-Demerdash et al. 2005), has successfully been used to predict possible hepatotoxicity or oxidative damage to functional liver membrane architecture (Mukinda and Eagles 2010; Nathwani et al. 2005). ALP is a critical index in bile duct obstruction and hepatic necrosis (Witthawaskul et al. 2003). In this study (Table 2), elevation noted in the serum activities of these biomarkers could possibly confirm ROS-induced hepatopathy inherent to DM (Nkosi et al. 2005; Ameson and Brickell 2007). However, several studies have reported increased activities of these hepatic biomarkers in hyperglycemic patients (Marchesini et al. 2001; Klonoff et al. 2008), insulin resistance and metabolic syndromes (Wannamethee et al. 2013). Nonetheless, the administration of Mg(OH)$_2$NP-$Mm$ observably reduced the activities of these membrane-bound enzymes. This possibly suggests the hepatoprotective potential of the biosynthesized particles, perhaps by stabilizing membranous integrity of hepatic tissue, and thereby protecting against deleterious effects of STZ-stimulated oxidative stress in diabetic hepatopathy (Madkour 2012).

Similarly, bilirubin, the end product of heme catabolism in the systemic circulation (Vítek 2012), a useful index in the excretory function of hepatocytes (Rigato et al. 2005; Puppalware et al. 2012). However, an increase was noted in the plasma bilirubin of STZ-induced diabetic, this could probably confirm DM-related hepatic impairment (Horsfall et al. 2012; Cai et al. 2004). However, a reduction noted in the bilirubin following treatment with the biosynthesized Mg(OH)$_2$NP-$Mm$ suggests the hepatoprotective activity of the particles possibly by enhancing excretory functions of hepatic tissue and thereby, protecting the hepatocytes from oxidative damage in DM-related hepatopathy.

In addition, a decrease in serum total protein levels in the diabetic untreated group (Table 2), might be as a result of an established hypoproteinaemia and hepatocellular dysfunction, which have been well implicated in DM and associated complications (Igbakin and Oloyede 2009). Several reports on DM have also connected this defect to an alteration in protein and free amino acids metabolism and synthesis in the liver (Gaskill et al. 2005), as well as muscle wasting and formation of protein-malondialdehyde (MDA) adducts (Sheela et al. 2013; Abou-Seif and Youssef 2004; Skov et al. 1999). However, an increase noted in serum TP following treatment with the biosynthesized Mg(OH)$_2$NP-$Mm$, could possibly suggest that, Mg(OH)$_2$NP-$Mm$ possess ability to control excessive protein catabolism, as well as reducing formation of protein-malondialdehyde adducts that have been
connected to DM and associated complications (Wolff et al. 1991). This finding agrees with the report of Luan et al (2021) and Ghanbari et al (2016).

Furthermore, plasma elevation of creatinine and urea have successfully been used as good indicators of renal dysfunction implicated in DM (Gowda et al. 2010). Creatinine is metabolic waste product primarily excreted by the kidneys (Huang et al. 2002). Urea plays an important role in the metabolism of nitrogen-compounds in animals and its serum concentration is influenced by excretion rate of the kidneys (Knepper and Roch–Ramel 1987; Levey et al. 1999). In this study (Table 3), elevated serum creatinine and urea levels were revealed in the STZ-induced diabetic control. The modification effect of compromised glucose metabolism on nitrogen homeostasis has been implicated in elevated hepatic dismissal of urea (Sabir et al. 2019; Dominik and Stange 2021). However, a reduction in glomerular filtration rate induced by renal injury has been reported to be responsible for urea and creatinine retention (Webster et al. 2017). On the other hand, elevated serum creatinine might have resulted from ketosis or ketoacidosis, following elevated serum acetoacetate implicated in DM (Westerberg 2013; Qiu et al. 2017). Administration of synthesized Mg(OH)$_2$NP-$Mm$ revealed no reduction in creatinine levels of the treated groups, however, a reduction in urea levels was noticed following treatment with Mg(OH)$_2$NP-$Mm$. The slight increase observed in creatinine levels after treatment with Mg(OH)$_2$NP-$Mm$, could indicate inability of the nanoparticles to fully enhance renal creatinine clearance (Yu et al. 2016). Whereas, a reduction in urea level probably suggests renoprotective potential of Mg(OH)$_2$NP-$Mm$, perhaps by enhancing renal urea clearance, as well as, its ability to protect the kidneys against STZ-induced nephropathy in the diabetic groups (Amartey et al. 2015).

Oxidative stress plays an important role in the pathogenesis of a number of human diseases (Liguori et al. 2018). However, antioxidant enzymes, the first line of cellular defense offer protection against deleterious effects of ROS in organisms (Adwas et al. 2019; Ighodaro and Akinloye 2018). Progressive depletion of endogenous antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), protein and non-protein thiols (glutathione peroxidase; GPx and reduced glutathione; GSH) have been implicated in hyperglycemia (Dawson and Storey 2017; Pisoschi et al. 2020). In this study (Figures 1, 2 & 3), a decline was observed in the SOD, CAT, protein and non-protein thiols levels of the hepatic, renal and brain tissues of STZ-induced diabetic animals in comparison with the normal group. Reduction of antioxidant system in these tissues probably corroborates STZ-induced oxidative stress, which could be substantiated with a noticeable increase in malondialdehyde (MDA), primarily a biomarker of lipid peroxidation, as well as various morphological modifications in the architectures of tissues (Figures 4, 5, & 6) (Baltacıoğlu et al. 2014; Ishaq et al. 2014). Nonetheless, this effect could have been elicited as a result of spontaneous generation of superoxide ($O_2^{-}$) free radical, a sole mechanism underlying the diabetogenic activity of STZ (Shairibha and Rajadurai 2014; Bhattacharya and Sil 2018).

However, following treatment with Mg(OH)$_2$NP-$Mm$, an increase was actuated in CAT, SOD, protein thiols, non-protein thiols, and total protein. An increase in thiol-dependent antioxidant proteins of the hepatic, renal and brain tissues among the treated groups, suggest the ability of the particles to donate hydrogen ion ($H^+$) via hexose monophosphate (HMP) pathway prompting the formation of NADPH+$H^+$, thus causing the stabilization of thiol-dependent proteins as supported by the reports of Henderson (1998), Wu et al (1996) and Ithayaraja (2011). Overall, given the increase, there is a clear indication that $Mm$-Mg(OH)$_2$Ns possesses an ability to mitigate against proliferation of ROS in these tissues, thus protecting them against oxidative injury prompted by
STZ-induced ROS (Di et al. 2016; Turkmen et al. 2019). However, this effect could be validated with the noticeable repairs in the histomorphological studies on the tissues as evident in Figures 4, 5, & 6. This report is in agreement with the findings of Ahmed et al (2020) and Barakat (2020).

**Conclusion**

Report of this study hypothesized activities demonstrated by the biosynthesized magnesium based nanomaterials (Mg(OH)$_2$Ns-$Mm$), such as restoration of normal glucose homeostasis, enzymatic activities and attenuation of oxidative stress capable of perturbing the integrity of hepatic, renal and brain tissues in STZ-induced diabetes. Therefore, given these activities, the biosynthesized Mg(OH)$_2$Ns-$Mm$ could probably be protective against diabetes-associated hepatopathy, nephropathy and neuropathy.

**Declarations**

**Conflict of Interest**

Authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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Tables

1: Effect of synthesized Mg(OH)$_2$Ns- $Mm$ on fasting blood glucose in STZ-induced diabetic rats

| Groups                          | Day 1             | Day 3             |
|--------------------------------|-------------------|-------------------|
|                                | Pre-Diabetic (mg/dl) | After 48 h (mg/ml) |
| After treatment (mg/ml)        |                   |                   |
| Normal control                 | 82.00 ±6.26       | 64.33 ±2.40       |
|                                | 81.56 ±19.94      |                   |
| Diabetic control               | 81.00 ±2.92       | 311.33 ±10.47*    |
|                                | 386.20 ±8.45*     | 386.20 ±8.45*     |
| Diabetic + 50 mg/kg Mg(OH)$_2$Ns-$Mm$ | 81.67 ±2.71       | 295.67 ±9.01*    |
|                                | 346.33 ±25.16*    | 346.33 ±25.16*    |
| Diabetic + 100 mg/kg Mg(OH)$_2$Ns-$Mm$ | 92.17 ±3.53       | 262.20 ±10.4*    |
|                                | 342.75 ±81.74*    | 342.75 ±81.74*    |
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-$Mm$ | 76.83 ±4.35       | 289.83 ±39.90*   |
|                                | 298.00 ±43.04***  | 298.00 ±43.04***  |
| Diabetic + 200 mg/kg Mg(OH)$_2$Ns-$Mm$ | 95.33 ±3.68       | 297.50 ±18.63*   |
|                                | 133.50 ±20.50**   | 133.50 ±20.50**   |
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-STD | 85.33 ±2.68       | 297.17 ±25.88*   |
|                                | 202.00 ±59.63***  | 202.00 ±59.63***  |
| Diabetic + 5 mg/kg Glibenclamide | 94.00 ±2.82       | 306.00 ±38.89*   |
|                                | 346.33 ±28.37*    | 346.33 ±28.37*    |

The values are expressed as means ± SEM of replicates (n=6) in each group. Different superscripts indicate data comparison down the column; * indicates p <0.05 compared to normal control; **p < 0.05 compared to diabetic control and p > 0.05 compared to normal control, while ***p < 0.05 compared to diabetic control and p < 0.05 compared to normal control.
Table 2: Effect of synthesized Mg(OH)$_2$Ns-Mm on serum ALP, AST, ALT, bilirubin and total pro (TP) in STZ-induced diabetic rats

| Groups                     | Bilirubin (µmol/L) | TP (mg/dl) | ALP (U/l)   | AST (U/l)   | ALT (U/l) |
|----------------------------|--------------------|------------|-------------|-------------|-----------|
| Normal control             | 8.33 ±0.79         | 1.62 ±0.13 | 98.62 ±8.15 | 29.00 ±1.15 | 8.45 ±1.03 |
| Diabetic control           | ±1.31*             | ±0.68 ±0.32* | 276.55 ±11.49* | 63.13 ±3.05* | 38.75     |
| Diabetic + 50 mg/kg Mg(OH)$_2$Ns-Mm | ±1.62* | ±0.90 ±0.34* | 227.70 ±13.31* | 54.00 ±3.85* | 34.25     |
| Diabetic + 100 mg/kg Mg(OH)$_2$Ns-Mm | ±6.33* | ±1.41 ±0.38 | 178.99 ±41.00*** | 63.13 ±2.40* | 36.27     |
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-Mm | ±2.44** | ±1.61 ±0.10 | 192.51 ±20.35*** | 35.00 ±4.81** | 18.95     |
| Diabetic + 200 mg/kg Mg(OH)$_2$Ns-Mm | ±7.50*** | ±1.24 ±0.10 | 151.66 ±4.00*** | 55.25 ±12.25* | 23.30     |
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-STD | ±3.12** | ±1.49 ±0.43 | 149.87 ±20.56*** | 53.88 ±4.93* | 16.80     |
| Diabetic + 5 mg/kg glibenclamide | ±3.00* | ±1.06 ±0.25 | 208.11 ±5.40* | 60.38 ±3.30* | 27.28     |

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

Table 3: Effect of synthesized Mg(OH)$_2$Ns-Mm on serum creatinine and urea in STZ-induced diabetic rats
| Groups                              | Creatinine (mg/ml) | Urea (mg/dl) |
|------------------------------------|--------------------|--------------|
| Normal control                     | 0.62 ±0.51         | 34.66 ±4.88  |
| Diabetic control                   | 1.48 ±0.07*        | 84.68 ±9.38  |
| Diabetic + 50 mg/kg Mg(OH)$_2$Ns-Mm| 1.82 ±0.20*        | 90.05 ±7.26* |
| Diabetic + 100 mg/kg Mg(OH)$_2$Ns-Mm| 2.73 ±0.13*       | 83.92 ±11.37*|
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-Mm| 1.82 ±0.13*       | 85.49 ±12.91*|
| Diabetic + 200 mg/kg Mg(OH)$_2$Ns-Mm| 1.70 ±0.11*       | 73.56 ±16.79*|
| Diabetic + 150 mg/kg Mg(OH)$_2$Ns-STD| 0.80 ±0.32**      | 77.24 ±11.45*|
| Diabetic + 5 mg/kg Glibenclamide   | 1.40 ±0.21*        | 126.50 ±3.64*|

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

**Figures**

**Figure 1**

(i-vi): Effects of the synthesized Mg(OH)$_2$NP-Mm on catalase (CAT), superoxide dismutase (SOD), MDA, total protein (TP), protein thiol (PT) and non-protein thiol (NPT) of hepatic tissue in STZ-induced diabetic rats.

The values are expressed as means ± SEM of six trials (n=6). * indicates a significant difference at p < 0.05 vs normal control, #p < 0.05 vs diabetic control, ##p < 0.05 vs diabetic control & p < 0.05 vs normal control. Note: PT, protein thiol; NPT, non-protein thiol, CAT, catalase; SOD, superoxide dismutase; MDA, malonyldehyde; TP, total protein.
Figure 2

(i-vi): Effects of the synthesized Mg(OH)$_2$NP-$Mm$ on catalase (CAT), superoxide dismutase (SOD), MDA, total protein (TP), protein thiol (PT) and non-protein thiol (NPT) of renal tissue in STZ-induced diabetic rats.

The values are expressed as means ± SEM of six trials (n=6). * indicates a significant difference at $p < 0.05$ vs normal control & # $p < 0.05$ vs diabetic control. Note: PT, protein thiol; NPT, non-protein thiol; CAT, catalase; SOD, superoxide dismutase; MDA, malonyladehyde; TP, total protein.
(i-vi): Effects of the synthesized Mg(OH)$_2$NP-Mm on catalase (CAT), superoxide dismutase (SOD), MDA, total protein (TP), protein thiol (PT) and non-protein thiol (NPT) of brain tissue in STZ-induced diabetic rats.

The values are expressed as means ± SEM of six trials (n=6). * indicates a significant difference at p < 0.05 vs normal control & #p < 0.05 vs diabetic control. Note: PT, protein thiol; NPT, non-protein thiol, CAT, catalase; SOD, superoxide dismutase; MDA, malonaldehyde; TP, total protein.
Figure 4

(1-8): Photomicrographs of hematoxylin-eosin staining of hepatic tissues (x800).

(1) Normal control shows normal architecture of liver cells (hepatocytes) and portal triad; (2) Diabetic control and (3) Diabetic + 50 mg/kg Mg(OH)$_2$Ns-$Mm$ reveal shrunk portal area and necrosis; (4) Diabetic + 100 mg/kg Mg(OH)$_2$Ns-$Mm$ shows normal architecture of hepatocytes and normal portal area; while (5) Diabetic + 150 mg/kg Mg(OH)$_2$Ns-$Mm$ shows mild disruption of the liver cells (hepatocyte); (6) Diabetic + 200 mg/kg Mg(OH)$_2$Ns-$Mm$ reveals shrunk portal area; (7) Diabetic + 150 mg/kg Mg(OH)$_2$-STD and (8) Diabetic + 5 mg/kg glibenclamide show normal hepatocyte with poor lobular organization. **Keys:** H, hepatocytes; portal area/triad (portal vein, hepatic artery and bile duct).
Figure 5

(1-8): Photomicrographs of hematoxylin-eosin staining of kidney tissues (x800).

(1) Normal control shows normal Bowman's capsule with normal urinary space, proximal convoluted tubules and distal convoluted tubules; (2) Diabetic control reveals expansion and disruption of glomerulus; (3) Diabetic + 50 mg/kg Mg(OH)_2_Ns-Mm, (4) Diabetic + 100 mg/kg Mg(OH)_2_Ns-Mm and (5) Diabetic + 150 mg/kg Mg(OH)_2_Ns-Mm show recovery of the glomeruli and convoluted tubules; (6) Diabetic + 200 mg/kg Mg(OH)_2_Ns-Mm reveal absence of urinary space; while (7) Diabetic + 150 mg/kg Mg(OH)_2-STD and (8) Diabetic + 5 mg/kg glibenclamide show normal glomerulus and convoluted tubules. Keys: G, glomerulus; US, urinary space/Bowman's space; PCT, proximal convoluted tubules; DCT, distal convoluted tubules.
Figure 6

(1-8): Photomicrographs of hematoxylin-eosin staining of brain tissues (x800).

(1) Normal control shows evenly distributed normal neuronal cells; (2) Diabetic control reveals pyknotic and scantily distributed neuronal cells; (3) Diabetic + 50 mg/kg Mg(OH)₂Ns-Mm, and (4) Diabetic + 100 mg/kg Mg(OH)₂Ns-Mm reveal scantily distributed and mild disrupted neuronal cells; (5) Diabetic + 150 mg/kg Mg(OH)₂Ns-Mm and (6) Diabetic + 200 mg/kg Mg(OH)₂Ns-Mm show presence of normal neuronal cell body; (7) Diabetic + 150 mg/kg Mg(OH)₂-STD, and (8) Diabetic + 5 mg/kg glibenclamide reveal recovery of neuronal cells.

**Key:** Black arrows indicate the neurons.