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Oral magnesium supplementation ameliorates glucose tolerance in streptozotocin-nicotinamide induced diabetic rats.

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Abstract

Magnesium supplementation has recently attracted attention as effective in the management of diabetes and its related complications though its mechanisms of action is yet to be fully unraveled. This study was carried out to determine the effects of magnesium supplementation on body weight, fasting blood sugar, Oral glucose tolerance (OGTT 2 and OGTT 4), glucose transporter isoform 2 (GLUT2), GLUT4 and insulin receptor (INSR) mRNA expressions in streptozotocin-nicotinamide induced diabetic *Sprague dawley* rats. A total of 24 *Sprague dawley* rats (Four groups of six rats each) were used for this study, and the treatment was for 28 days. Group 1: Normal control rats were given distilled water; Group 2: Metformin treated rats were given 100 mg/kg body weight; Group 3: Metformin + Magnesium treated rats were given 100 mg/kg and 1000 mg/kg body weight respectively; Group 4**:** Diabetic untreated rats given distilled water. Data were analyzed statistically using Analysis of Variance (ANOVA) and graphically by Graph pad prism. The GLUT4 and INSR gene expressions of Group 3 were significantly ($p<0.05$) upregulated when compared with Group 4. There was significant ($p<0.05$) decrease fasting blood sugar and GLUT2 mRNA level in the treated diabetic rats but the metformin-magnesium supplement treated group showed more decrease ($p<0.05$) when compared with the group treated with metformin only. This study demonstrates that magnesium may mediate effective metabolic control by stimulating insulin sensitivity, and upregulating mRNA levels of GLUT4, INSR as well as improving glucose tolerance in diabetic rats. Therefore, magnesium supplementation appears to have a beneficial role and improves glucose uptake by cells in those at high risks of diabetes.

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Keywords: *Magnesium supplementation, Metformin, INSR, GLU4, glucose tolerance.*

1.0 Introduction

Diabetes mellitus is a group of metabolic disorder that affects more than 100 million people worldwide (6% population). It is characterized by a chronic hyperglycemic/high blood sugar condition resulting from a deficiency in production of insulin hormone responsible for the elimination of high blood of 5mM or above (type 1 diabetes mellitus) or insulin resistance/insulin sensitivity (type 2 diabetes mellitus) [Deshmukh and Jain, 2015; Harikumar, *et al.,* 2015].

Type 2 diabetes mellitus is also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes mellitus. It is characterized by insulin resistance/sensitivity in insulin target tissues such as liver, skeletal muscle and adipocytes [Morakinyo *et al.,* 2018; Fapohunda and Balogun, 2019]. Insulin resistance has been shown to cause a decline in the sensitivity of the insulin receptor to Insulin [Morakinyo *et al.,* 2018].

Magnesium is one of the essential minerals associated with glucose transport across the cell membrane and essential in energy homeostasis [Feng *et al.,* 2019; Morakinyo *et al.,* 2018]. It is required as a cofactor for many enzymatic reactions of carbohydrate metabolism, protein synthesis as well as the second messenger for insulin action [Ebel and Gunther 1980; Reinhart 1988; Solaimani *et al.,* 2014]. It has been shown that magnesium ions are distributed distinctly in various organs and tissues in different proportion, 50-65% of the total body magnesium are found in the bone, 25% in skeletal muscle, the remaining are found in other tissues like liver and heart [Valk, 1999]. 50-60% of magnesium in the blood is in the free state, ionized form while the remainder is bound to protein or anion like phosphate, citrate and oxalate.

Magnesium deficiency is implicated in the development of chronic complication in type 2 diabetes (T2D) [Hans *et al.,* 2002; Solaimani *et al.,* 2014; Fapohunda and Balogun, 2019]. Intracellular magnesium plays a crucial role in regulating insulin, phosphorylation of insulin receptor kinase and insulin-mediated glucose uptake [Barbagallo and Dominguez, 2015; Gunther 2011; Sari *et al.,* 2000; Volpe 2013].

Puri *et al.,* 2013 reported that a reduction in intracellular magnesium concentration results in postreceptorial impairment in insulin action and worsening of insulin resistance in a diabetic patient. Deficiency in magnesium ion and reduced insulin sensitivity occur as a result of the

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presence of oxidative stress and inflammation, which lead to elevation of free radicals in type 2 diabetes patients [Nasreen *et al.*, 2018]. Recent studies have suggested that increased Mg intake is associated with lower fasting glucose and insulin level as well as a lower risk of T2D [Dong *et al.*,.2011; Hruby *et al.*, 2013; Morakinyo *et al.*, 2018].

GLUT2 and GLUT4 are families of sodium/glucose transporter isoforms (SGLT) expressed in liver and skeletal muscle, respectively. GLUT2 and GLUT4 translocation from an intracellular pool to the plasma membrane occur when insulin binds insulin receptor, which is usually expressed on the surface of insulin sensitivity tissues to clear the high blood sugar 5mM or above from the plasma [Leto and Saltiel, 2012]. Hence this study was designed to show the efficacy of oral magnesium supplement on the body weight, fasting blood sugar, glucose tolerance, insulin receptor, GLUT2 and GLUT4 in streptozotocin-nicotinamide induced diabetic rats.

2.0 MATERIALS AND METHOD

2.1. Diabetic Models

All protocols related to animal studies were approved by the Animal Ethics Committee of Centre for Research and Development Adekunle Ajasin University Ondo State, Nigeria. Twenty-four (24) male *Sprague dawley* rats (average weight of 150 g) were obtained from the Department of Plant Science and Biotechnology, Adekunle Ajasin University Ondo State, Nigeria. They were housed under standardized environmental conditions (well-ventilated room, with 12-hour light-dark cycles and 55 ± 4 % at 24 ± 2 ^{o}C). Animals were allowed to feed *ad libitum.* They were divided into groups (n=6) based on their weight which was used to calculate the dosage of Streptozotocin (STZ, Sigma Aldrich, Hamburg, Germany), Nicotinamide (NAD), Magnesium Sulfate (MgSO4, Sigma Aldrich, Hamburg, Germany), and Metformin (Merck pharma care spoxil) administered. Induction was carried out after three weeks of acclimatization. The administration started when the rats were confirmed diabetic after 72 hours of induction. The intervention was carried out daily in the following order for four (4) weeks:

Group 1: Normal control rats were given distilled water.

Group 2: Metformin treated rats were given 100 mg/kg body weight.

Group 3: Metformin + Magnesium treated rats were given 100 mg/kg and 1000 mg/kg body weight respectively.

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Group 4**:** Diabetic untreated rats given distilled water.

2.2 Methods

2.2.1 Induction of diabetes in rats

NIDDM was induced as described by Venkateshwarlu *et al.,* (2015) with little modification. Briefly, overnight-fasted rats were given intraperitoneal injection (i.p.) of freshly prepared 60 mg/kg STZ (dissolved in a citrate buffer of pH 4.8), 5 minutes after the i.p. administration of 110 mg/kg of freshly prepared NAD dissolved in normal saline.

2.2.2 Intervention

Magnesium sulfate and metformin were intubated into the mouth of the diabetic rats in quantity based on their body weights.

2.2.3 Oral glucose tolerance test (OGTT)

At the second and fourth week, the oral glucose tolerance test was carried out. Blood was collected from the tip of the tail of the animals with a surgical blade. It was applied to the blood glucose strip that was inserted into the glucometer. The animals were subjected to fast for 16 hours before the oral glucose tolerance test. The body weights of animals were measured and recorded just before taking the fasting blood glucose level on the prepared record sheet. The tails of the rats were snipped as they were placed on the table. Then small incision was made over their lateral vein (1-2cm from the tail tip). Fasting blood glucose levels of the animals were determined before glucose administration with the aid of glucose strips inserted in a glucometer. 2 g/kg body weight of sucrose was freshly prepared in distilled water and administered orally with the aid of an oral cannula. Before the administration of glucose, fasting blood glucose level was measured (baseline). The blood glucose level was also repeatedly measured at 30, 60,120 and 180 minutes after glucose administration.

2.2.4 Sacrificing and tissue excision

At the end of the treatment, the animals were subjected to fasting overnight for 9 hours and to cervical dislocation following ethical care and handling of experimental animals' regularities, and they were dissected using dissecting set. The rats were sacrificed, and the gastrocnemius muscle and liver were excised from each experimental animal. Little quantities of the excised tissues were dropped in Eppendorf tubes containing 0.2 µl TRIzol across the groups and then spun using laboratory centrifuge.

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2.2.6 Gene expression profiling

RNA was isolated from the pancreas using TRIzol Reagent (ThermoFisher Scientific) following the manufacturer's guide. Purified DNA-free RNA was converted to cDNA immediately using ProtoScript® First Strand cDNA Synthesis Kit (NEB). PCR amplification was done using the following primer set:

Table 1:

TARGET GENE	FORWARD 5'-3'	REVERSE 3'-5'
GAPDH	TGA AGG TCG GAG TCA ACG GAT TTG GT	CAT GTG GGC CAT GAG GTC CAC CAC
GLUT4	GCA ACA TGT CAG AAG ACA AGA TCA	TAG CTC TTC GGT CAT CCA GAG
GLUT2	GCA ACA TGT CAG AAG ACA AGA TCA	TAG CTC TTC GGT CAT CCA GA G
INSR	CTGCATCGGACTCTACCAGG	AGGGAAAGGCAGTGGGTCTA

A representative snapshot of reverse transcription-polymerase chain reaction-agarose gel electrophoresis data of all the rats were taken and analyzed using the band density (Image-J) which is then plotted as a bar graph (Mean ± SEM).

2.2.7 Statistical analysis

Data are expressed as mean± standard error of mean (SEM) and analyzed using the ANOVA followed by Tukey's Multiple Comparison post-hoc test. A p-value below 0.05 was considered statistically significant. (*) means significant difference $p<0.05$ when compared with Group 1 (NORMAL).

(#) means significant difference $p<0.05$ when compared with Group 4 (DU).

(α) means significant difference $p<0.05$ when compared with Group 3

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3.0 RESULTS

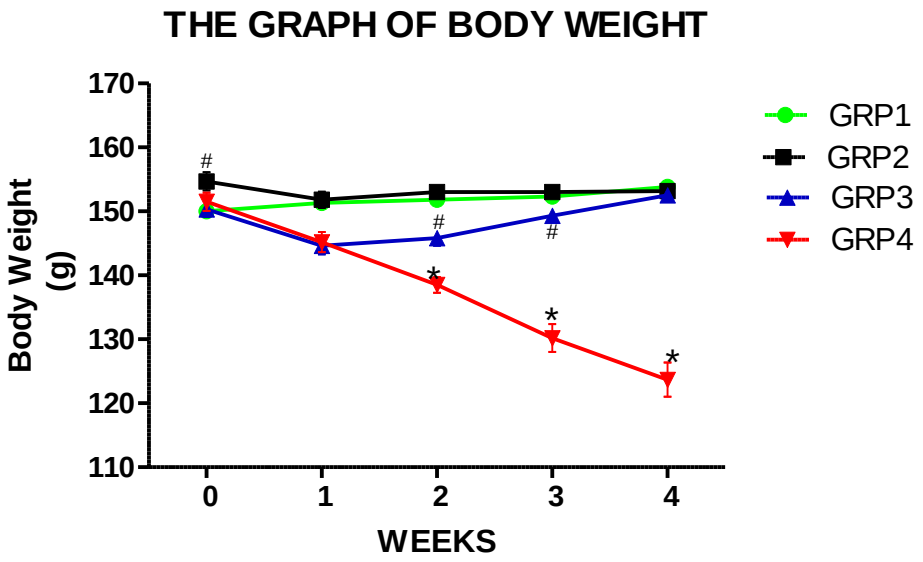

Figure 1: Effect of magnesium supplement on body weight.

3.1 EFFECT OF MAGNESIUM SUPPLEMENTATION ON BODY WEIGHT.

Figure 1 showed the effect of oral magnesium supplementation on body weight. It was observed that all the diabetic groups showed significant ($p<0.05$) reduction in body weight from week 0 to 1. The body weight of the rats in the NORMAL group, however, increased consistently from week 0 to week 4. There was a change in the trend of body weight of the diabetic groups after week 1 with Group 2 and Group 3 increasing to 152g and 150g respectively while the untreated diabetic Group 4 kept reducing in body weight till the end of the experiment.

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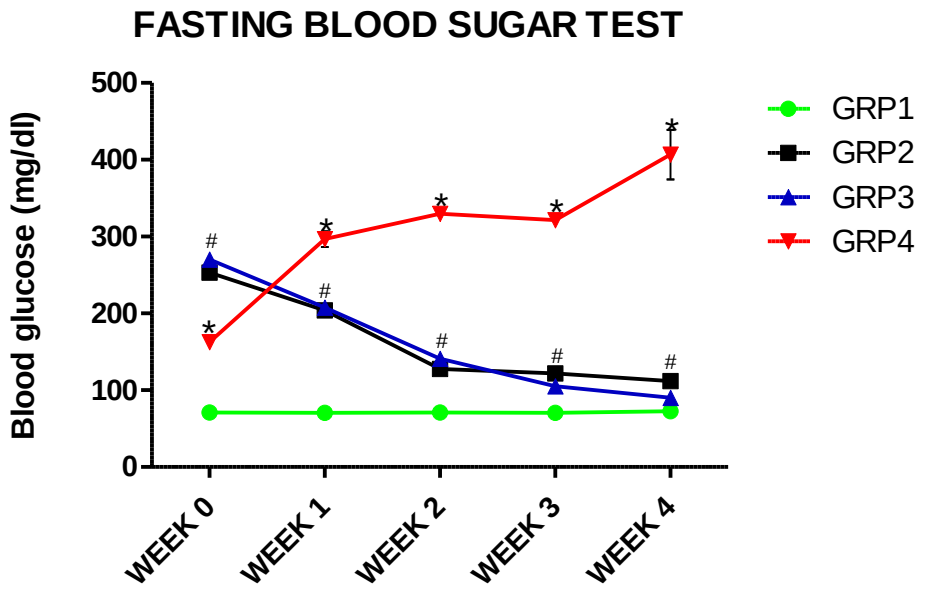

Figure 2: Effect of magnesium supplementation on Fasting blood sugar.

3.2 FASTING BLOOD SUGAR (FBS).

There was a significant ($p < 0.05$) increase in the FBS of Group 4 from week 0 to week 4 when compared with other groups. Group 2 and 3 showed a drastic and significant reduction in FBS when compared with Group 4. Group 1 relatively maintained its FBS level all through the period of the experiment. However, the reduction in FBS of Group 3 (199.33%) was more significant when compared with that of Group 2 (126.07%).

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3.3 ORAL GLUCOSE TOLERANCE TEST AND PHARMACOKINETICS

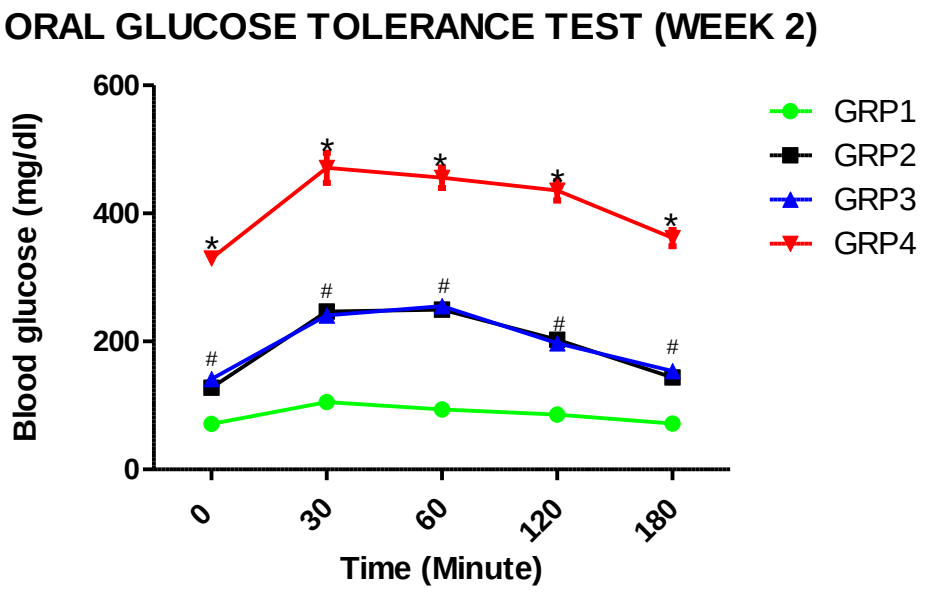

Fig 3: Effect of Magnesium supplementation on glucose tolerance test in diabetic rats. (Week 2)

EFFECTS OF MAGNESIUM SUPPLEMENTATION ON GLUCOSE TOLERANCE IN DIABETIC RATS (OGTT 2)

There was a significant increase in Groups 2, 3 and 4 when compared with Group 1. Also, there was a reduction in Groups 2 and 3 when compared with group 4. However, there was no significant difference between Groups 2 and 3

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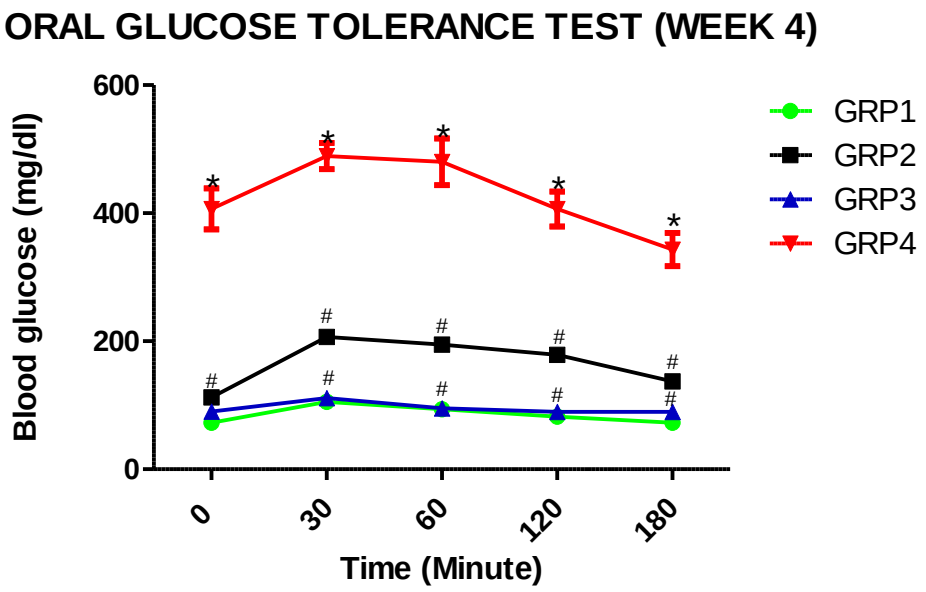

Fig 4: Effect of magnesium supplementation on oral glucose tolerance test in Diabetic rats (week4)

EFFECT OF MAGNESIUM SUPPLEMENTATION ON GLUCOSE TOLERANCE IN DIABETIC RATS (OGTT 4)

There was a significant difference between Groups 2 and 4 when compared with Groups 1 and 3. However, there was a significant increase in Group 4 when compared with Group 2. Nevertheless, there was no significant difference between Group 1 and Group 3.

Regarding the pharmacokinetics of glucose obtained from all the groups, the C_{max} value for the untreated diabetics increased tremendously at week 2 (471.0 mg/dl) and week 4 (489.3 mg/dl) with t_{max} (30 minutes) respectively, whereas the C_{max} value of the treated Group 2 and Group 3 reduced from 249.5 mg/dl and 255.2 mg/dl respectively at 60 minutes to 206.7 mg/dl and 111.0 mg/dl at 30 minutes respectively.

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Table 2: Pharmacokinetics parameters of magnesium supplementation glucose tolerance in diabetic rats (week 2)

GROUPS	GROUP 1	GROUP 2	GROUP 3	GROUP 4
C_{max} (mg/dl)	105.2±1.47	249.5 ± 10.35	255.2 ± 2.67	471.0 ± 23.67
t_{max} (min)	30.0	60.0	60.0	30.0
AUC (C*t)	3156.0 ± 1.47	14970.0 ± 10.35	15312.0 ± 2.67	14130.0 ± 23.67

Table 3: Pharmacokinetics parameters of magnesium supplementation on oral glucose tolerance test in Diabetic rats (week 4)

GROUPS	GROUP 1	GROUP 2	GROUP 3	GROUP 4
C_{max} (mg/dl)	105.3 ± 2.60	206.7 ± 3.28	111.0 ± 2.58	489.3 ± 20.31
t_{max} (min)	30	30	30	30
AUC (C*t)	3159 ± 2.60	6201± 3.28	3330 ± 2.58	14679 ± 20.31

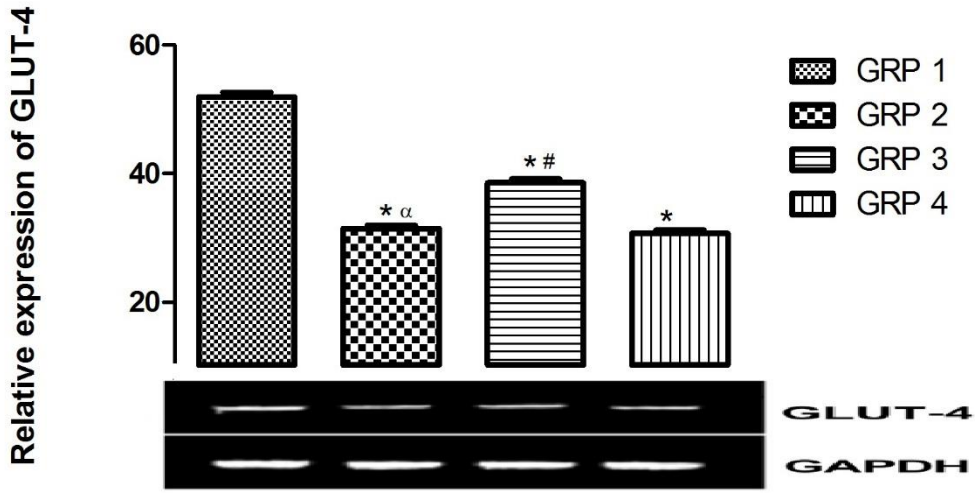

Fig 5: Effect of magnesium supplementation on GLUT4 gene in Gastrocnemius muscle

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3.4 GLUT4 GENE EXPRESSION IN THE SKELETAL MUSCLE

From Figure 5, the level of GLUT4 gene expression in skeletal muscle samples was quantified in gastrocnemius muscle, GLUT4 was significantly downregulated in Groups 2, 3 and 4 when compared with Group 1. However, there was no significant difference between Group 2 and 4, but the GLUT4 gene expression level was significantly higher in Group 3 when compared with Group 2 and Group 4.

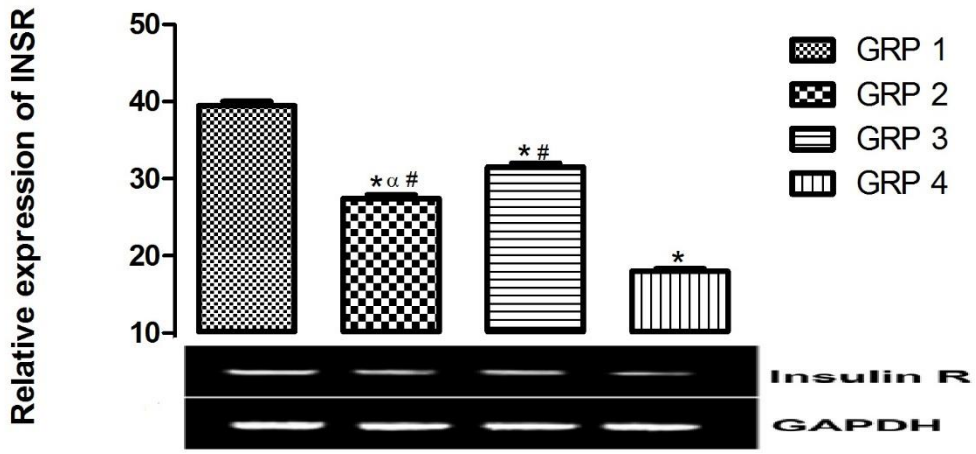

Fig 6: Effect of magnesium supplementation on Insulin Receptor (INSR)

3.5 INSR GENE EXPRESSION IN THE SKELETAL MUSCLE

From Figure 6, the level of INSR gene expression in skeletal muscle samples was quantified in gastrocnemius muscle, INSR was significantly downregulated in Groups 2, 3 and 4 when compared with Group 1. However, significant upregulation was seen in Groups 2 and 3 when compared to Group 4. Nevertheless, Group 2 was significantly lower when compared with Group 3.

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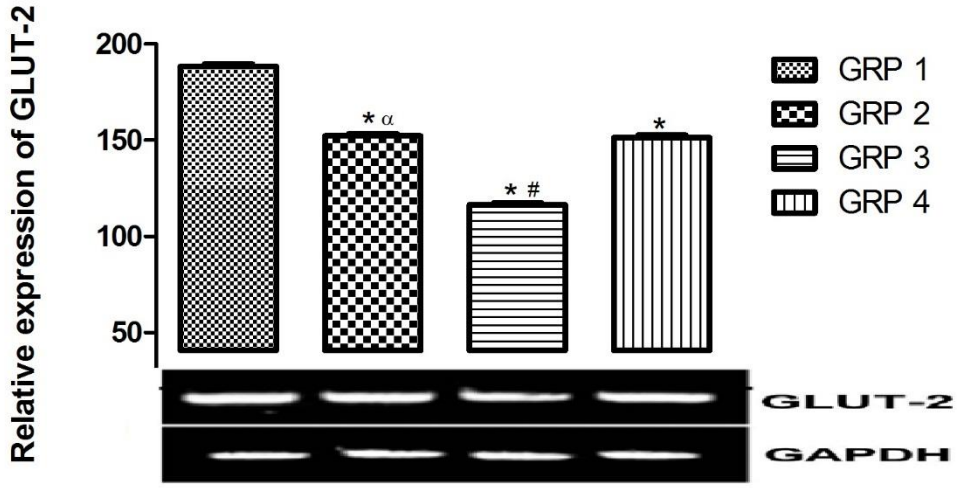

Fig 7: Effect of magnesium supplementation on GLUT2.

3.6 GLUT2 GENE EXPRESSION IN THE LIVER

From Figure 7, the level of GLUT2 gene expression was quantified in the liver, GLUT2 was significantly high in the diabetic Groups 2, 3 and 4. Meanwhile, significant downregulation of GLUT2 mRNA expression was observed in Group 3 when compared with Group 2 and 4 at the end of the treatment. However, there was no significant difference between Groups 2 and 4.

4.0 DISCUSSION

Magnesium is one of the essential mineral elements of significant advantage in the human body system. It aids the activity of many metabolic pathways including protein synthesis and DNA production, it acts as a cofactor of various metabolic enzymes and also activates various signaling pathways in the body system [Gimenez-Mascarell *et al.,* 2018; Grober *et al.,* 2015; Costello *et al.,* 2016; de Baaij *et al.,* 2015; Morakinyo *et al.,* 2018; Solaimani *et al.,* 2014].

Magnesium deficiency is implicated in the development of chronic complication in type 2 diabetes (T2D) [Hans *et al.,* 2002; Fapohunda and Balogun, 2019; Fapohunda 2018]. Magnesium use up/exhaust has been shown to have an antagonistic effect on glucose homeostasis and insulin sensitivity in patients with T2D by altering the gene expression of the diabetic biomarker/or enzymes [Thomas and Philipson, 2015, Fapohunda and Balogun, 2019]. Hence, in this present study, we aim to examine whether the magnesium-metformin adjunct altered GLUT2, GLUT4 and INSR genes and how this contributes to improved glucose homeostasis in the Dawley diabetic rats.

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From the result in figure 1, there was a drastic reduction in the body weight of the untreated diabetic group from week 0 to week 4 (22.47%). This pattern might have been due to the characteristic weight loss of adipose tissue [Ozata *et al.,* 2001; Matthaei *et al.,* 2000]. The treated diabetic groups showed a reduction in body weight from week 0 to week 1, but there was an increase in the body weight after week 1 from 145g to 155g and from 152g to 155g for the magnesium-metformin adjunct group and the metformin-treated group respectively. This pattern may be due to ability of metformin to recuperate the beta pancreatic cell and magnesium to restore the abnormal metabolic pathways associated with diabetes [Omotayo *et al.,* 2011; Feng *et al.,* 2019]. In the overall, the metformin-treated Group 2 showed 0.98% body weight reduction at the end of week 4 with no significant difference from the normoglycemic Group 1. This finding is not out of place as some studies have earlier reported that treatment of diabetic rats with metformin dosages of 25mg/kg and 100mg/kg could cause more loss in body weight and no inhibitory effect on body weight reduction respectively [Campbell *et al.,* 1994; Fontbonne *et al.,* 1996; Lee and Morley, 1998; Pouya *et al.,* 2012]. In the same vein, the metformin-magnesium co-treated Group 3 plunged in body weight from week 0 (before diabetes induction) to week 1. This pattern was however reversed and at the end of week 4 with 1.46% increase in body weight from week 0 to week 4.

Meanwhile, this increase is not significantly different from normoglycemic Group 1, which showed a 2.5% increase in body weight from week 0 to week 4. This finding bears credence to the fact that magnesium supplement, when used with metformin, has a positive combinatorial effect on weight management in T2D as suggested my Fapohunda, (2018). This result is in tandem with the findings of Omotayo and his team (2011) who reported that metformin had a positive combinatorial effect on body weight when used with honey.

It has also been shown that diabetes associate with destruction of beta-cell/insulin sensitivity resulting in decreased in insulin production or insulin resistance leading to dysfunction in glucose metabolism and is evidently as impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) [Jaja and Okoh 2015; Festa *et al.,*. 2006; Morakinyo *et al.,* 2018]. Impaired glucose has been identified using plasma glucose level between 140-200mg/dl during a 2hour oral glucose tolerance test. After the point of diabetes induction at week 0, the FBS level of all the diabetic groups was significantly higher than the normoglycemic Group 1, according to figure 2. This finding is as a result of STZ-NAD, the diabetogenic agent used which must have

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caused partial destruction of the β-cells of the pancreas leading to impaired insulin secretion leading to hyperglycemia (Fapohunda *et al.,* 2020; King, 2012; Marchetti *et al.,* 2017). This trend continued for untreated diabetic Group 4 till the end of the experiment. However, Groups 2 and 3 showed a drastic and significant reduction in FBS when compared with Group 4 but, the reduction in FBS of Group 3 (199.33%) was more significant when compared with that of Group 2 (126.07%). This result shows that magnesium supplementation exerted more hypoglycemic effect when used in combination with metformin than when metformin is used alone. This consistent with other studies (Fapohunda *et al.,* 2020; Barbagallo and Dominguez, 2015; Lal *et al.,* 2003) and may be ascribed to magnesium's documented potentiation of insulin secretory activities, its glucose regulatory and blood glucose stabilizing effects. Control Group 1 relatively maintained its FBS level all through the period of the experiment.

Before glucose loading, all groups except the Normal Control Group 1 showed a high level of blood glucose when fasted overnight. The Diabetic Untreated Group exhibited a significant ($p<0.05$) increase in blood glucose level at all points compared with other Groups and also failed to return to their time-zero value within the 180 minutes' post glucose loading. This result indicated that the rats were hyperglycemic in line with the report of Morakinyo *et al.,* (2018), Tahara *et al.,*. (2008); Mahmoud *et al.,* (2012). Although glucose challenge significantly ($p<0.05$) raised the glucose level of Group 2 and Group 3 compared with Group 1 at 30 and 60 minutes; however, these Groups show noticeable improvement in oral glucose tolerance test as the blood glucose was remarkably lower than the Diabetic Untreated Groups at all points at week 2 and the glucose tolerance improved significantly ($p<0.05$) in Group 3 than Group 2 at week 4. This leads to the deduction that magnesium supplementation may help to improve insulin sensitivity consequently regulate blood glucose. This is in synchrony with the reports of Wester, (1987); Singh *et al.,* (2010); Fapohunda, (2018).

The results above in tables 2 and 3 show the bioavailability of glucose during week 2 and week 4, respectively. The result during week 2 showed there was a significant increase of maximum concentration and area under the curve of the plasma glucose in diabetic treated groups and untreated group of when compared with normal group. However, the maximum concentration of glucose was lower in the diabetic treated groups when compared with diabetic untreated. Also during week 4 the result followed a similar trend as week 2. However, there was a significant reduction in metformin-magnesium treated group trying to bring maximum concentration and

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area under the curve of plasma glucose to the level of normal group. This was made possible because magnesium has a specific site on the receptor, where it binds to execute its regulatory effect on plasma glucose [Dingledine *et al.,* 1999]. However, the bioavailability of metformin absorption is nonlinear, and it is excreted unchanged in the urine because it does not undergo hepatic metabolism [Dell'Aglio *et al.,* 2009; Kazory *et al.,* 2007; Schwartz *et al.,* 2006; Najib *et al.,* 2002].

Metformin is one of the significant drugs considered in type 2 DM therapy. It improves glucose metabolism and diabetes-related complications. Rena *et al.,* (2017) reported that metformin can aid the reduction of glucose production in the liver and expression of resistin genes. Moreover, metformin can increase the GLUT4 gene expression (Wu *et al.,* 1998). An increase in GLUT4 gene expression aids insulin sensitivity (Aligita *et al.,* 2020). In the same vein, it was hypothesized that magnesium helps in modulating blood glucose by upregulating GLUT4 mRNA expression (Solaimani *et al.,* 2014). GLUT4 is a family of sodium-glucose transporter isoforms predominantly found in adipose and skeletal tissues. Insulin signaling is activated by the binding of insulin hormone with the insulin receptors located on the plasma membrane. This result in the phosphorylation of tyrosine that acts as a a binding site for insulin receptor substrate protein leading to the employment of phosphatidylinositol-3-kinase to outer cell [Furtado *et al.,*. 2002; Watson *et al.,*. 2002; Backer *et al.,* 1992; Scheid *et al.,* 2002]. Hence resulting in the translocation of GLUT4 from GLUT4 storage vesicles (GSVs) in the intracellular environment to the cell surface, thereby facilitating the glucose uptake from the blood for other processes like glycogenesis [Kumar *et al.,* 2010]. In this study, the GLUT4 gene expression of the diabetic untreated group was significantly downregulated when compared with the Group 3 treated with metformin and magnesium. This is similar to previous reports where fasting, high-fat feeding, obesity and diabetes were identified to aid lowering the GLUT4 mRNA concentration in adipose and skeletal muscle tissue (Eckardt *et al.,* 2011; Tremblay *et al.,* 2001). In a research carried out by Kang and colleagues (2013), it was demonstrated that supplementation of antidiabetic drugs with *Opuntia humifusa* (a clump-forming cactus which is rich in magnesium and other essential minerals) leads to up-regulation of glucose uptake by increasing PPAR-γ, PGC-1α and GLUT4 protein expressions, this enables insulin sensitivity in rat skeletal muscle. It was also revealed in another study that the GLUT4 protein expression was significantly reduced in the skeletal muscle and adipose tissue of type 2 DM patients (Kampmann *et al.,* 2011). The result in figure 5

shows the upregulation of GLUT-4 mRNA expression in the magnesium-metformin adjunct group when compared with other treated groups. This could be made possible as a result of up-regulation of the insulin receptors activated by intracellular magnesium leading to the reduction in blood glucose, consequently translocation of GLUT4 from intracellular to the cell surface. Hence facilitating the glucose clearance from the plasma [Leto and Saltiel 2012], which is line with the report of Solaimani *et al.,* (2014) Han *et al.,* (2002) Volpe, (2013) Morakinyo *et al.,* (2018). *In vivo* studies have shown that knockout of GLUT4 in the muscle of mice results in the development of diabetes [Zisman *et al.,*. 2000; Abel *et al.,*. 2001, Bell *et al.,* 1990].

The insulin receptor (INSR) is a tyrosine kinase receptor, it is a transmembrane receptor that is predominantly activated by insulin, insulin-related growth factors, IGF-1 and IGF-2, and some insulin analogs (Ward *et al.,* 2009; Lawrence, 2009). Metibemu *et al.,* (2019) expounded the structure of INSR being a subfamily of receptor tyrosine kinases. Ligand binding to the two insulin-binding α-subunit of INSR causes dimerization of the extracellular domain leading to a conformational transformation that stimulates the intracellular domain at the two β-subunit causing autophosphorylation at the tyrosine residues [Feng *et al.,* 2019; Gutiérrez-Rodelo *et al.,* 2017]. The autophosphorylation of the insulin receptor follows the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway (White, 1998) and RAS/RAF/MEK/ ERK pathway (Ward *et al.,* 2009). The tyrosine kinase activity at the two β-subunit of INSR is dependent on $Mg^{2+,}$ which acts as its cofactor (Hruby, 2013). In a diabetic condition, defect in INSR leads to impairment in insulin-induced activation of insulin receptor substrate (IRS)/PI3K/Akt pathway leading to a reduction of insulin-stimulated GLUT4 trafficking and glucose uptake in skeletal muscles (Lee *et al.,* 2017; Shanik *et al.,* 2008; Boura-Halfon and Zick, 2009; Jiang *et al.,* 1999). In this present study, it was observed that INSR gene expression was significantly downregulated in an uncontrolled diabetic state. However, when magnesium supplement was administered along with the standard drug, metformin, INSR gene expression was upregulated in gastrocnemius muscles of diabetic rats. These revealed that magnesium might mediate effective metabolic control by regulating the expression of INSR and GLUT4 in diabetic rats. This result is in concordance with previous research carried out by Morakinyo *et al.,* (2018), although their findings focused on protein expression of INSR and GLUT4. It also agreed with the report of

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Chen *et al.,* (2014) that Mg pretreatment could aid the expression of GLUT4, enhancing the transfer of glucose to the muscles during exercise.

GLUT2 is the family of glucose transporter protein/a member of the SLC2A gene family, expressed in liver, intestine, kidney and pancreatic islet beta-cell that mediate the uptake glucose. GLUT2 is required for the control of glucose-sensitive genes; therefore, hepatic uptake and release of glucose depends on it (Narasimhan *et al.,* 2015). Its inactivation in the liver leads to impaired glucose-stimulated insulin secretion [Leturque *et al.,* 2005]. GLUT2 is a bidirectional transporter with relatively high Km for glucose. It maintains intracellular glucose homeostasis with that of blood. The nutritional and hormonal environment usually modulates GLUT2 protein and mRNA level. Increased level of GLUT2 protein and mRNA usually accompany postprandial hyperglycemia and type 2 diabetes (Im *et al.,* 2005). We, therefore, studied the effect of metformin-magnesium combination therapy on type 2 diabetic animal model. From figure 7, the mRNA expression level of GLUT2 was high in diabetic rats, but it was significantly downregulated in the metformin-magnesium treated group. This suppression may be caused by the presence of unsuspected glucose output pathway that may depend on membrane traffic-dependent mechanism. Similar results were reported when by Narasimhan *et al.,* (2015) and Villanueva-Peñacarrillo *et al.,* (2001) when they administered ferulic acid and GLP-1 respectively.

CONCLUSION

It is interesting to discover from this present study that metformin-magnesium adjunct therapy has antidiabetic potency by improving glucose tolerance and regulating essential genes (GLUT2, GLUT4 and INSR) involved in glucose homeostasis. However, the detailed mechanistic approach of the combination therapy needs to be unveiled. It is, therefore, imperative for researchers to further investigate the effect of this therapy on critical transcriptional factors that affect the mRNA expression of the genes in this present study. For instance, the following factors which affect GLUT4 mRNA expression viz: myocyte enhancer factor 2A (MEF2A), glucose enhancer factor (GEF), CCAAT/enhancer-binding protein (C/EBP), PPARγ, hypoxia-inducible factor 1α (HIF-1α), sterol regulatory element-binding protein 1c (SREBP-1c), Krüppel-

like factor 15 (Klf15), nuclear factor 1 (NF1) and histone deacetylase 5 (HDAC5). In the same vein, SREBP-1c, Hepatocyte nuclear factor 1α (HNF1α), HNF4α, HNF3β (FoxA2), C/EBP, C/EBPβ all regulate GLUT2 gene expression.

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