

Effect of Magnesium pre-treatment on Alloxan induced hyperglycemia in rats

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Abstract

Background: The role of vitamins and mineral supplementation in the prevention of diabetes mellitus is not well elucidated.

Objective: The effect of prior administration of magnesium on alloxan induced diabetes was assessed in rats.

Methods: 36 Male albino rats were used for this study. The animals were divided into 6 groups of 6 animals each; group 1 was healthy control; groups 2 served as diabetic control. Animals in group 3 received magnesium (100 mg/kg) i.p one hour prior to alloxan (120 mg/kg) administration, group 4 were also received magnesium (150 mg/kg) i.p one hour prior to alloxan administration. Animals in group 5 received magnesium (100 mg/kg) i.p only; group 6 animals received magnesium (150 mg/kg) i.p only. Blood samples were obtained from all animals and plasma glucose levels were determined on Day 0 (prior to treatment), Day 2, Day 5, Day 7 and Day 10 after the commencement of treatment.

Results: There was significant increase ($P < 0.001$) in plasma glucose values in the alloxan treated group when compared with the control values. There was also a significant increase ($P < 0.01$) in plasma glucose levels in the magnesium-pretreated (100 mg/kg and 150 mg/kg) diabetic groups when compared with healthy controls whereas there was a significant reduction ($P < 0.01$) in plasma glucose level when compared with the diabetic control.

Conclusion: This study has shown that magnesium pretreatment may delay the onset and subsequently cause a reduction in hyperglycemia in alloxan induced diabetes. This effect of magnesium may be attributed to its role as a scavenger of highly reactive hydroxyl radicals generated through alloxan reactions, its potentiation of glutathione antioxidant production and its role as a calcium blocker.

Keywords: Magnesium, Hyperglycemia, Alloxan

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Introduction

Diabetes is a chronic disease characterized by elevated blood glucose level and disturbances in carbohydrate, fat and protein metabolism. There are two main categories of this disease, Type 1 diabetes mellitus also called insulin-dependent diabetes mellitus (IDDM) and Type 2, the non-insulin dependent diabetes mellitus (NIDDM).

IDDM represents a heterogenous and polygenic disorder, with a number of non-HLA loci contributing to the disease susceptibility¹. There is yet no identified agent substantially capable of preventing this type of disease².

NIDDM is far more common and results from a combination of defects in insulin secretion and action. Treatment of Type 2 diabetes is complicated by several factors inherent to the disease process, typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization³.

Vitamins and minerals play diverse roles in the body. They most commonly function as essential coenzymes and cofactors for metabolic reactions and thus help support basic cellular reactions (i.e., glycolysis, the citric acid cycle, lipid and amino acid metabolism) required to maintain energy production and life^{4,5}. They are also important in the regulation of metabolism, gene expression and may influence the development and progression of many chronic diseases.

A number of studies have reported an association between diabetes mellitus (DM) and (100

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Impaired insulin release, insulin resistance and glucose intolerance in experimental animals and humans with DM have been linked to a compromised status of chromium, magnesium, selenium, vanadium and zinc⁶. Some of these minerals (e.g., zinc, chromium, magnesium) are excreted at higher than normal rates in patients with DM, often leading to excessive urinary mineral wasting^{4,5}.

Magnesium functions as an essential cofactor for more than 300 enzymes. It is essential for all energy-dependent transport systems, glycolysis, oxidative energy metabolism, biosynthetic reactions, normal bone metabolism, neuromuscular activity, electrolyte balance, and cell membrane stabilization⁶. Poor control of diabetes has often been associated with low serum magnesium⁷. Magnesium deficiency has also been found to be common in children with type I diabetes⁸. The reduced concentrations of magnesium seen in diabetics appears to result in part from increased urinary magnesium excretion⁷.

Although a reduction in insulin release has been reported in individuals with compromised magnesium status, most of the focus on magnesium supplementation in DM is directed at preventing long-term complications. There is however a dearth of information regarding the prevention of the onset of diabetes by magnesium. It was previously reported that pre-administration of Magnesium may delay the onset of hyperglycemia in alloxan diabetes⁹. However, whether this delay was temporary, prolonged or permanent was not elucidated. This study therefore examined the effect of pre-administration of magnesium on alloxan diabetes over a 10 day period.

Methods

Chemicals and Reagents

Magnesium sulphate (Mg_2SO_4) and alloxan was purchased from Sigma Aldrich Chemical Co. (St. Louis, Missouri). 100 mg of Mg^{2+} (as Mg_2SO_4) was dissolved in 1ml of distilled water to give a stock solution of 100 mg/ml and this was thereafter administered to the experimental animals at single doses of 100 mg/kg and 150 mg/kg¹⁰ respectively intraperitoneally. 100 mg of alloxan granules were dissolved in 1ml normal saline to make a stock solution of 100 mg/ml and this administered after an overnight fast at a single dose of 120 mg/kg body weight intraperitoneally¹¹.

alterations in the metabolism of several minerals^{4,5}. **Animals**

Thirty-six male albino rats weighing between 160g – 250g were used in this study. All the animals were acclimatized in the Animal House of the Physiology Department, College of Medicine, University of Ibadan for 14 days prior to any procedure. Animals were housed in well aerated cages, fed on standard rat pellets obtained from Ladokun feeds and allowed free access to drinking water.

Animal Grouping

Animals were divided into six (6) groups of six animals each as follows:

- I. Healthy control group
- II. Diabetic control group
- III. Magnesium 100 mg/kg pretreated group
- IV. Magnesium 150 mg/kg pretreated group
- V. Magnesium 100 mg/kg only group
- VI. Magnesium 150 mg/kg only group

Induction of Diabetes Mellitus

Diabetes was induced with a single intraperitoneal dose of alloxan (120 mg/kg body weight)¹¹ after an 18 hour overnight fast. Thereafter, the animals were allowed free access to food and water.

Plasma glucose levels were determined using the glucose oxidase method¹². The level of blood glucose considered to be normal in *rattus norvegicus* ranges from 50 mg/dl – 135 mg/dl¹¹. In this study, rats with glucose levels above 250 mg/dl were considered as having severe diabetes.

Experimental protocol

The experimental protocol followed is as described by Heikkila and Cabbot¹³. Male albino rats weighing between 160 – 250g were obtained from the College of Medicine animal house for this study. The rats were kept in the departmental animal house for 2 weeks to stabilize. They were then fasted for 18 hours after which basal blood glucose levels were determined using blood from the tail vein of each rat.

The 36 rats were divided into 6 groups of 6 rats each. Animals in group 1 were normal and given single doses of 0.2 ml of 0.9% NaCl intraperitoneally. They served as healthy control. Animals in group 2 were made diabetic with alloxan (120 mg/kg) and did not receive any pretreatment pre or post diabetes, these served as diabetic control. Animals in group 3 were pretreated with magnesium

(120 mg/kg) administration. Animals in group 4 were also pretreated with magnesium (150 mg/kg) intraperitoneally one hour prior to alloxan (120 mg/kg) administration. Animals in group 5 were given intraperitoneal injection of magnesium (100 mg/kg) only, while animals in group 6 were treated with 150 mg/kg magnesium only. The blood glucose levels from all groups were monitored for a ten day period with blood samples obtained from the tail vein on day 0, day 2, day 5, day 7 and day 10.

mg/kg) intraperitoneally one hour prior to alloxan **Statistical analysis**
Results were expressed as the mean \pm SEM. Statistical comparisons were made using Student's t Test. Level of significance were evaluated at P= 0.05, 0.01 and 0.001 and differences were regarded as statistically significant when P values were 0.05 or less.

Results

The results of all the experiments carried out are shown in tables 1 – 2 and figures 1

Table 1: Body weight changes in control and experimental animals

Group/Day	Day 0 weight (g)	Day 2 weight (g)	Day 5 weight (g)	Day 7 weight (g)	Day 10 weight (g)
Healthy control	170.00 \pm 3.65	172.00 \pm 4.28	175.17 \pm 2.01	178.83 \pm 3.74	178.00 \pm 4.47
Diabetic control	188.33 \pm 5.43	166.67 \pm 4.94	148.33 \pm 7.03	131.67 \pm 5.43	120.00 \pm 3.65**
Magnesium (100mg/kg) pretreated diabetic group	216.67 \pm 6.15	191.67 \pm 4.01	160 \pm 0.00	160 \pm 0.00	148.33 \pm 4.77**
Magnesium (150mg/kg) pretreated diabetic group	175.00 \pm 3.42	151.67 \pm 3.07	136.67 \pm 8.03	116.67 \pm 4.22	115.00 \pm 3.42**
Magnesium (100mg/kg) only	230.00 \pm 7.30	205.00 \pm 7.19	220.00 \pm 5.16	228.33 \pm 9.10	238.33 \pm 7.93
Magnesium (150mg/kg) only	203.33 \pm 4.03	180.00 \pm 3.83	193.33 \pm 5.89	198.33 \pm 7.46	206.66 \pm 5.85

Values are mean \pm SEM.

** = P \leq 0.01 significant difference between day 0 values and day 10 values within the group

Table 2: Percentage change in Blood glucose levels in control and experimental animals on day 10 when compared with day 0

Group	Day 10
Healthy Control group	0%
Diabetic Control group	408.33% \pm 11.36%***
Magnesium (100mg/kg) pretreated diabetic group	351.94% \pm 4.36%***##
Magnesium (150mg/kg) pretreated group	236.46% \pm 11.03%*** ##
Magnesium (100mg/kg) Only Group	22.27% \pm 2.27%
Magnesium (150mg/kg) Only Group	7.95% \pm 1.61%

Values are percentages differences between day 0 and day 10 values.

*** = P \leq 0.001 Significant difference between healthy control values and experimental values;

= P \leq 0.01 significant difference between magnesium pretreated diabetic group and diabetic control.

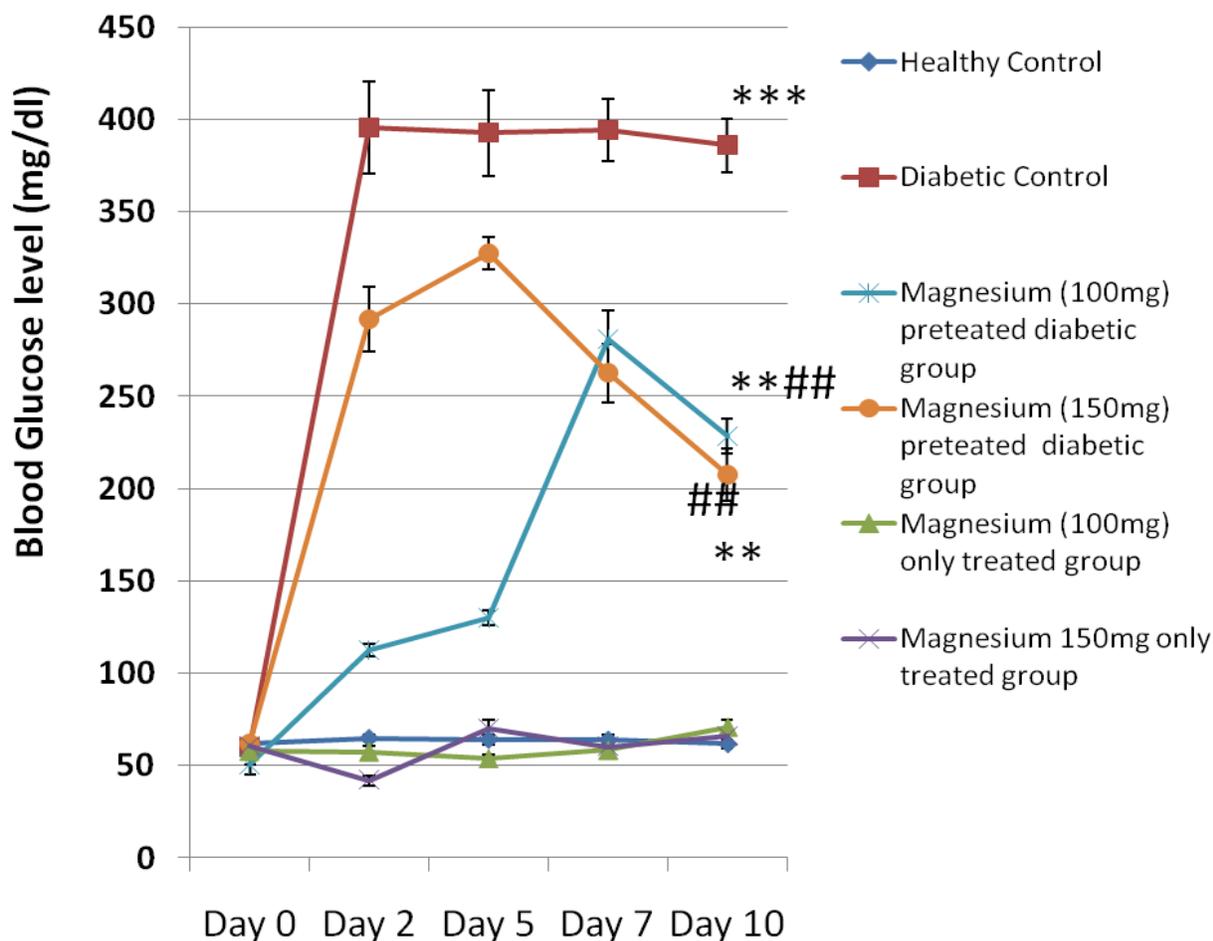
Blood Glucose Levels

Figure 1 shows the blood glucose level in control and experimental animals from day 0 to day 10. There was significant increase (P<0.001) in fasting plasma glucose values in the diabetic untreated group on Day 2 (395.5 \pm 24.78 mg/dl), Day 5 (392.5 \pm 23.05 mg/dl), Day 7 (394.17 \pm 16.65 mg/dl) and Day 10 (385.83 \pm 14.57 mg/dl) when compared with the control values. There was also a significant increase (P<0.01) in plasma glucose levels in the magnesium-pretreated (100 mg /kg and 150 mg/kg) diabetic groups when compared with the healthy control group. However, there was a significant reduction (P<0.01) in blood glucose level in the magnesium pre-treated diabetic groups on Day 2, Day 5, Day 7

and Day 10 when compared with the diabetic controls. The values obtained in the magnesium 100 mg/kg pretreated alloxan group on the days above are as follows 112.5 \pm 3.1 mg/dl, 130 \pm 3.87 mg/dl, 281.17 \pm 15.1 mg/dl, 228.33 \pm 9.5 mg/dl while values obtained in the magnesium 150 mg/kg pretreated alloxan group are as follows 291.83 \pm 17.79mg/dl, 327.5 \pm 8.92mg/dl, 262.5 \pm 15.8 mg/dl, 207.5 \pm 14.13 mg/dl.

It may be observed that plasma glucose level in the magnesium 150 mg/kg pretreated group declined after day 5 while that of the magnesium 100 mg/kg treated group declined after day 7.

Figure 1: Blood glucose level in control and experimental animals



***= $P \leq 0.001$ significant difference between experimental values and control values across the groups;
 **= $P \leq 0.01$ significant difference between experimental values and control values across the groups;
 ## = $P \leq 0.01$ significant difference between diabetic control animals and magnesium pretreated animals

Body weight changes

Table 1 shows the body weight changes in control and experimental from day 0 to day 10. It may be observed that there was an insignificant increase in the body weight in the control group by day 10 when compared with day 0 values, while in the diabetic group there was a steady significant decline ($p < 0.05$) in the body weight of the diabetic animals. There was a 31.6% and 34.3% reduction in the body weights of magnesium pretreated diabetic animals which was not significantly different 36.3% decrease in the diabetic group.

In the magnesium only groups (100 mg/kg and 150 mg/kg) there was an increase in the body weight on day 10 when compared with day 0 values. This increase in body weight was however statistically insignificant.

Discussion

In this study it was observed that prior administration of magnesium appeared to delay the onset of hyperglycemia as observed in alloxan diabetes and thereafter caused a gradual decline in hyperglycemia in the experimental animals. It was also observed that there was decrease in body weights of the rats in the diabetic group as well as in the magnesium pretreated diabetic groups and this appears to be consistent with the previous report of World Health Organization, that diabetes mellitus is often characterized by rapid and significant weight loss leading to fatigue which is not easily reversed¹⁴.

Alloxan administration has been reported to lead to necrosis of pancreatic beta cells. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals with subsequent dismutation to hydrogen

peroxide. This leads to formation of highly reactive hydroxyl radicals by the Fenton reaction. One of the targets of the reactive oxygen species is DNA of pancreatic islets¹⁵. DNA damage by the reactive oxygen species lead to cell death by necrosis.

Magnesium ions are essential to the basic nucleic acid chemistry of life, and thus are essential to all cells of all known living organisms. Plants have an additional use for magnesium in that chlorophylls are magnesium-centered porphyrins. Many enzymes require the presence of magnesium ions for their catalytic action, especially enzymes utilizing ATP or those which use other nucleotides to synthesize DNA and RNA. Recently there has been growing interest in magnesium deficiency and its correlation with coronary artery disease, chronic complications of diabetes mellitus and antioxidant enzyme activity¹⁶. Magnesium has also been reported to be an obligatory cofactor in GSH synthesis and in all biosynthetic reactions involving ATP¹⁷. In animals with reduced glutathione levels it has been reported that Mg²⁺ pretreatment was efficient in restoring renal and testis GSH levels in tissues of mice exposed to acute and subacute cadmium intoxication¹⁸.

It has been reported that GSH is the main intracellular free radical scavenger and is known to provide protection against free radicals¹⁹. It has been reported to divert hydrogen peroxide from the pathway leading to the formation of hydroxyl radicals²⁰. In addition to this Sakurai and Ogiso observed that the *in vitro* generation of hydroxyl radicals in the presence of alloxan strongly depends on GSH concentration¹².

The reduction in plasma glucose level in the magnesium pretreated group when compared with the diabetic group may be due to the ability of magnesium to increase the activity of GSH within the pancreatic beta cells. The increased GSH activity could have caused an increase in the scavenging of free radicals produced by alloxan administration.

It has also been proposed that disturbances in intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan. This concept was confirmed by *in vitro* and *in vivo* experiments demonstrating that alloxan elevates cytosolic free Ca ion concentration in pancreatic B cells^{22,23}.

The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by hydrogen peroxide since it has been reported that

hydrogen peroxide exerts a similar effect on calcium concentration in B cells²³. The exaggerated concentration of calcium ion leads to supraphysiological insulin release and, together with reactive oxygen species, causes damage of pancreatic B cells¹¹.

Magnesium has also been shown to be a physiologic calcium blocker²⁴. It has been reported to inhibit calcium overload through inhibition of calcium transport across most calcium channels²⁵. Hence calcium overload caused by alloxan administration may have been prevented, reduced or delayed as a result of the effects of magnesium as a calcium channel blocker.

Conclusion

It may be deduced from this study that pretreatment with magnesium may cause a delay in the onset and subsequent decline in hyperglycemia as observed in alloxan diabetes. This effect of magnesium may be due to the free radical scavenging ability of magnesium, its ability to potentiate antioxidant glutathione production or its physiological action as a calcium channel blocker.

It is suggested that further studies be carried out in order to evaluate the effect of magnesium intake on the blood glutathione level as well on the plasma level of other free radical scavengers.

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