Effect of Vitamin D on Blood Sugar, HbA\textsubscript{1c} and Serum Insulin Levels in Streptozotocin-Induced Diabetic Rats

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\textbf{BACKGROUND}

\textbf{Background:} Type 2 diabetes mellitus (T2DM) is an increasingly common disorder characterized by chronic hyperglycemia and marked dyslipidemia. This study evaluates the effect of vitamin D supplementation alone and in combination with glimepiride in streptozotocin-induced T2DM in rats.

\textbf{Materials and methods:} A total of 30 Wistar albino rats of either sex weighing 150-200 g were included in the study. The effect of oral administration of vitamin D was evaluated in streptozotocin-induced T2DM in rats. Blood glucose, serum insulin, serum HbA\textsubscript{1c}, and serum vitamin D were evaluated.

\textbf{Results:} Vitamin D treatment has significantly improved hyperglycemia, hyperinsulinemia, and insulin sensitivity compared with the non-treated diabetic rats. Oral administration of vitamin D in streptozotocin-induced T2DM reduced blood sugar levels, increased insulin levels (more prominently when administered along with glimepiride) and decreased HbA\textsubscript{1c} levels (p<0.005).

\textbf{Conclusion:} Administration of vitamin D can improve hyperglycemia and hyperinsulinemia in streptozotocin-induced T2DM in rats. Thus, it could be considered as an add on therapy along with other antidiabetic drugs.

\textbf{Keywords:} type 2 diabetes mellitus, streptozotocin, HbA\textsubscript{1c}, antidiabetic drugs.

\textbf{INTRODUCTION}

Type 2 diabetes mellitus (T2DM) is a multifactorial disease such as chronic hyperglycemia, altered insulin secretion, and insulin resistance – a state of decreased responsiveness to normal concentrations of circulating insulin (1, 2). It is also defined by impaired glucose tolerance (IGT) that results from islet β-cell dysfunction, followed by insulin deficiency in skeletal muscle, liver, and adipose tissues (3, 4). In individuals with IGT, the development of T2DM is governed by genetic predisposition and environmental variables (a hypercaloric diet and the consequent visceral obesity, or increased adiposity in liver and muscle tissues) and host-related factors (age, imba-
lances in oxidative stress, and inflammatory responses) (5, 6). Clinical complications of T2DM include both microvascular diseases (e.g., retinopathy, nephropathy, and neuropathy) and macrovascular complications (e.g., myocardial infarction, peripheral vascular disease, and stroke) (1). Macrovascular complications are the leading cause of mortality among diabetic persons (7). Although important knowledge has been acquired on the etiology of diabetes, its precise etiopathogenesis is still under debate. Inflammatory factors, reactive oxygen species, and autoimmune reactions have all strongly emerged as the major pathogenic effectors for diabetes.

The involvement of vitamin D in pathogenesis and prevention of diabetes has recently generated widespread interest. Many studies highlighted a correlation between vitamin D and incidence of T2DM. Thus, a seasonal variation in glucose control was reported to be worse in winter in T2DM patients (4), which may be due to prevalent hypovitaminosis D as a result of reduced sunlight during the cold season. Insulin resistance is a recognized precursor for the development of T2DM. Vitamin D has a favorable effect on insulin action either directly – by stimulating the expression of insulin receptors and thereby, enhancing insulin responsiveness for glucose transport (7) – or indirectly via its role in regulating extracellular calcium, ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic Ca^{2+} pool. Calcium is important for insulin-mediated intracellular processes in insulin-responsive tissues (skeletal muscle and adipose tissue), with a very narrow range of Ca^{2+} needed for optimal insulin-mediated functions. Changes in Ca^{2+} in primary insulin target tissues may contribute to peripheral insulin resistance via impaired insulin signal transduction, leading to decreased GLUT-4 activity (7). It also was shown to improve glucose tolerance (2). Vitamin D, by its function as an important antioxidant, may also prevent T2DM.

It was found that few animal studies were showing the effect of vitamin D on blood sugar levels, especially in combination with oral hypoglycemic agents. Many exertions are shifted towards disease prevention and a search for safer drugs. Several studies showed that T2DM was associated with low vitamin D levels. For this reason, our study aimed to test the effect of vitamin D supplementation alone and along with an oral hypoglycemic agent (glimepiride) in T2DM. More precisely, the study objectives were to evaluate the effect of vitamin D supplementation alone and in combination with glimepiride on serum insulin, HbA1c, and blood glucose levels in streptozotocin (STZ) induced T2DM in rats receiving a high-fat diet (HFD).

**MATERIALS AND METHODS**

A total of 30 Wistar albino rats of both sexes, weighing 150-200 g, were included in the study. The work was carried at the central animal house of BLDE (Deemed to be University), Sri. B.M. Patil Medical College Hospital and Research Center, Vijayapur, Karnataka, India. Animals were kept at room temperature (22-28˚C), with 55±10% relative humidity, for 12 hours, being exposed to dark-light cycle, and were given standard pellet chow, HFD, and water ad libitum. Institutional Animal Ethics Committee (IAEC) approval was obtained (NO-01/BLDE(DU)/2018).

Streptozotocin was purchased from Sigma Aldrich USA. Glimepiride was procured from IPCA laboratories, Mumbai, and vitamin D was purchased from Fermenta Biotech. HFD was prepared as per Guo et al. (8). Insulin, HbA1c, and Vitamin D kits were purchased from Chongqing Biospes Co., Ltd, China.

**Experimental design**

Rats were divided into five experimental groups (n=6), each kept in a separate cage, as follows: group 1=control; group 2=diabetic control; group 3=diabetic control with vitamin D; group 4=diabetic with standard treatment (glimepiride); and group 5=diabetic with standard treatment + vitamin D.

Rats in groups 2-5 were fed with HFD till completion of the study (during three weeks), and STZ was administered intraperitoneally (IP) in a dose of 35 mg/kg (8) body weight (dissolved in distilled water). After five days, blood samples were tested for glucose levels, and values above 250 mg/dL were included in the study. Group 1 received liquid paraffin daily and standard pellet chow.

Post diabetes, rats in group 3 were given vitamin D 4 000 IU/kg body weight orally, while groups 4 and 5 received glimepiride and glimepiride + vitamin D, respectively, for three weeks. Glimepiride was administered in a dose of 100 mcg/kg body weight orally. Liquid paraffin
was used as a vehicle for the administration of vitamin D and glimepiride. At the end of the study, blood samples were collected through retro-orbital plexus using heparinized capillary tubes, centrifuged, and serum was used for estimation of insulin, HbA1c and vitamin D.

**RESULTS**

When compared to group 1, all rats had a significant gain in body weight (p<0.05), but in group 2, the gain in body weight has been not substantially increased, being rather constant after one week of study. At the same time, body weight had a significant decrease (p<0.05) in rats treated with glimepiride (group 4), glimepiride, and vitamin D (group 5) (Figure 1).

At baseline, blood glucose levels were comparable in all groups. In group 2, there was an increase in blood glucose levels (hyperglycaemic), which remained consistently high from week 1 to the end of the study; in groups 4 and 5, blood glucose levels were significantly (p<0.05) reduced; moreover, addition of vitamin D to the treatment given to group 5 rats lead to a marked reduction compared to glimepiride alone (Table 1).

Among diabetic control rats (group 2) there were decreased serum insulin levels and significantly increased HbA1c levels (p<0.05) compared to the control group 1, whereas vitamin D levels were comparable. Diabetic rats treated with vitamin D (group 3) showed a slight increase in serum insulin levels and a decrease in those of HbA1c compared to group 2, while vitamin D levels were significantly elevated (p<0.05). Glimepiride treatment (group 4) lead to an increase in serum insulin levels and a significant reduction in those of HbA1c (p<0.05), as compared to untreated diabetic rats (group 2). A significant increase in levels of serum insulin and vitamin D as well as a significant decrease in HbA1c, levels was seen when glimepiride and vitamin D were administered concomitantly (group 5) compared to group 2, and serum insulin values were comparable to group 1 (Table 2).

**DISCUSSION**

In this study, experimental induction of T2DM in rats resulted in increased fasting blood glucose and decreased fasting serum insulin. Also, vitamin D supplementation has significantly lowered glucose levels and raised insulin levels; this may be endorsed to supplementation with vitamin D which has been shown to reinstate insulin secretion in animals (9).

Our study showed that vitamin D treatment has significantly improved hyperglycemia, hyperinsulinemia, and insulin sensitivity compared with non-treated diabetic rats. The reduction in glucose levels was found to be significantly higher in combined therapy with vitamin D and glimepiride than glimepiride monotherapy. The improvement obtained with vitamin D supplementation was in agreement with the study of Zeitz et al (10). Others provided evidence that vitamin D supplementation exerted beneficial effects in obese spontaneously hypertensive rats and Wistar

![Body weight (g)](image)

**FIGURE 1.** Body weight (g) in all groups (mean±SD)

**TABLE 1.** Blood glucose levels (mg/dL) in all five study groups (mean±SD)
Effect of Vitamin D in Streptozotocin-Induced Diabetic Rats

Table 2. Serum insulin, HbA1c and Vitamin D levels (mean±SD)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Serum Insulin (mU/L)</th>
<th>HbA1c (nmol/L)</th>
<th>Vitamin D (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>7.65±0.25</td>
<td>718±91.04</td>
<td>244.81±24.02</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.7±0.53*</td>
<td>1426.5±110.03</td>
<td>267.66±19.52</td>
</tr>
<tr>
<td>Group 3</td>
<td>3.14±0.40</td>
<td>1299.3±83.32</td>
<td>393.5±23.17*</td>
</tr>
<tr>
<td>Group 4</td>
<td>5.8±0.46*</td>
<td>981±90.26*</td>
<td>300.16±20.76*</td>
</tr>
<tr>
<td>Group 5</td>
<td>6.66±0.58*</td>
<td>856±40.21*</td>
<td>386.33±30.42*</td>
</tr>
</tbody>
</table>

*p<0.05 compared to control, *p<0.05 compared to diabetic control.

In a study by Calle et al (16), treatment with vitamin D to streptozotocin-induced diabetic rats improved the decreased basal glucose transport by 107%, which was correlating to a reduction in glucose levels in our study. Treatment with vitamin D resulted in a noteworthy increase in insulin concentration, which could improve hyperglycemia in diabetic rats. Serum HbA1c has significantly decreased in vitamin D treated rats, which is consistent with a study by Hoda et al (17).

Control rats had constantly increased their body weight, whereas diabetic rats dropped body weight during the first week after STZ injection, probably because of decreased glucose metabolism and increased fat metabolism (18). Subsequently, the body weight of rats in the STZ group was further increased until the completion of the study, while the groups with vitamin D and glimepiride and glimepiride + vitamin D group did not have an increase in body weight similarly to diabetic control – there was a significant difference, and the p-value was <0.001. The authors considered that the reduced insulin and increased body weight induced by STZ had a higher resemblance to T2DM. The difference in body weight between group 2 and groups 4 and 5 was significant (p <0.001).

The possible mechanisms by which vitamin D can influence the metabolism of glucose may be the result of a rapid non-genomic effect or a slower genomic effect of vitamin D by stimulating insulin release through increased VDR expression (19). Another possible mechanism is the suppression of the release of proinflammatory cytokines that are believed to mediate insulin resistance. The latter hypothesis is supported by studies showing an association between low serum 25(OH)D and increased C-reactive protein levels. Besides, vitamin D may indirectly influence the extracellular and intracellular calcium regulation, which is essential in mediating glucose transport in target tissues. In an attempt to understand the role of vitamin D in β-cell function, Nyomba et al (20) found that plasma calcium levels, vitamin D binding protein (DBP), circulating vitamin D, and bone mass were reduced in STZ-induced diabetic rats. These defects were related to altered metabolism of vitamin D due to an inhibitory effect of insulin deficiency on renal function 25(OH)D3 1α-hydroxylase. The concentration of HbA1c decreased marginally, but insignificantly, in group 3, and had a significant decrease in groups 4 and 5. In addition, a conspicuous enhancement was observed in the insulin level. Previous studies suggest several different mechanisms to explain the role of vitamin D in normalizing the glucose level. Lack
of vitamin D has been shown to result in impairment (disturbance) of glucose metabolism by increasing insulin resistance, which in turn occurs due to decreased adipose expression and degradation of the β-cell function and mass (21).

Supplementation with cholecalciferol was found to restore the alteration in IP3 and AMPA receptor, a non-NMDA-type ionotropic transmembrane receptor for glutamate expression in the pancreatic islets, which helped to restore calcium-mediated insulin secretion, indicating the therapeutic role of vitamin through the regulation of glutamatergic function in diabetic rats (22).

CONCLUSION

Administration of vitamin D can improve hyperglycemia, hyperinsulinemia in streptozotocin-induced type 2 diabetes mellitus in rats, thus can be potentially considered as add on therapy along with other antidiabetic drugs. However, further randomized clinical control trials are required to confirm the dose and duration before it is included in the therapy of T2DM.

Conflicts of interest: none declared.

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REFERENCES


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