World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

The potential role of sitagliptin as an oral treatment regimen for type I diabetes mellitus

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Received: 06-04-2017 / Revised: 12-05-2017 / Accepted: 21-05-2017 / Published: 27-05-2017

ABSTRACT

Type 1 diabetes mellitus (T1D) has become an emerging worldwide epidemic with growing incidence all over the world in the past several decades. Insulin replacement has been the backbone of therapy in T1D which is characterized by a near total insulin deficiency. Current treatments still do not resemble the pancreatic β-cells' endogenous insulin profile, and all still show risks of hypoglycemia, ketosis and suboptimal control. The management of T1D remains an ongoing challenge. In this study, we investigated the potential role of *Sitagliptin* (*Sita*) as an oral treatment regimen for T1D using Streptozotocin (STZ) induced T1D model in rats. Daily oral administration of *Sita* (10 mg/kg) for 7 weeks significantly reduced blood glucose levels and HbA_{1c}. It increased serum insulin levels while decreasing serum glucagon and also showed an anti-oxidant activity by increasing GSH content and SOD activity with concomitant reduction of MDA and nitrite/nitrate contents. Immunohistochemical analysis showed an improvement in β-cells in treated groups when compared to diabetic one. **Conclusion:** *Sitagliptin* has the ability to improve the overall glycemic control in type 1 diabetic rats.

Keywords: Type 1 DM, β-cell, Regeneration, Sitagliptin

INTRODUCTION

Type 1 diabetes mellitus (T1D) has become an emerging worldwide epidemic with growing occurrence all over the world in the past several decades [1]. Since T1D is characterized by a near total insulin deficiency, insulin replacement has been the backbone of therapy. More physiologic insulin replacement approaches with newer insulin analog, and insulin pumps combined with continuous glucose monitoring systems have enhanced the ability to attain glycemic targets, however, the management of T1D remains an ongoing challenge. There is an increased risk of hypoglycemia and weight gain in case of tight control. Hypoglycemia is often known as the major barrier to improved glycemic control, as well fear of hypoglycemia can also contribute to failure in reaching glycemic targets [2].

Current treatments still do not resemble the pancreatic β-cells' endogenous insulin profile, and all still show risks of hypoglycemia, ketosis and suboptimal control [3]. Sitagliptin (*Sita*) is a reversible and competitive inhibitor of dipeptidyl peptidase-4 enzyme (DPP-4), resulting in about 90% inhibition of DPP-4 effect in plasma [4]. After meal, DPP-4 inhibitors increase active endogenous glucose dependent insulinotropic polypeptide (GIP) as well as glucagon like peptide (GLP-1) concentrations by 2 to 3 folds.

As long as hyperglycemia is present, this action results in enhancing insulin secretion as well as suppression of glucagon secretion. Though under hypoglycemic circumstances, counter regulatory mechanism of glucagon secretion by alpha cells is not compromised; on the contrary, using DPP-4 inhibitors may even enhance sensitivity of alpha cells to glucose [5]. *Sita* is approved as an adjunct to life style to enhance glycemic control in adults with type 2 diabetes mellitus (T2D) [6].

In T2D patients, administration of DPP-4 inhibitors improved β-cell function including Homeostasis Model Assessment–β (HOMA-β), proinsulin to insulin ratio and parameters of beta cell responsiveness in meal tolerance test. They also improved alpha cells' sensitivity to glucose, causing a more glucose dependent glucagon secretion [7].

This study investigated the potential effects of *Sita* in management of T1D rats.

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MATERIAL AND METHODS

Drugs and chemicals: Streptozotocin (STZ) was acquired from Sigma-Aldrich (St Louis, MO, USA). It was dissolved in 0.1 M citrate buffer (prepared by dilution of 10 ml of 100 mM citric acid with 90 ml distilled water and mix with 100 ml of 10 mM trisodium citrate; pH = 4.5 and stored at 4^{o}C) [8]. *Sita*: (PubChem CID: 4369359) was purchased from the market as 100 mg tablets, and was prepared as 0.1% suspension in 0.5% CMC.

Experimental animals: Adult male Sprague-Dawely rats (number = 30, weight = 210-230 g) were bought from the Holding Company for Biological Products and Vaccines (VACSERA), Agouza, Giza, Egypt. For accommodation, rats were housed six per cage at room temperature with 12 hours light/dark cycle for two weeks before starting the experiment. They were kept at constant environmental and nutritional settings all over the experimental period. The experimental procedures took place in this study consents to the ethical principles and rules for the care and utilization of laboratory animals established by the Faculty of Pharmacy Research Ethics Committee, Mansoura University.

Experimental design: Rats were distributed into 3 groups: Group I consisted of untreated rats (normal control) (n= 10). Group II served as untreated diabetic group (diabetic control) (n=10). Group III consisted of diabetic rats treated with *Sita* (n=10). Diabetes was induced in Group II and III, by single i.p. administration of 65 mg/kg STZ (dissolved in 0.1 M sodium-citrate buffer) and an equal amount of the sodium-citrate buffer was given to the normal control rats [9]. Ninety six hours after STZ injection, diabetes was confirmed by measuring blood glucose using a glucometer instrument (FreeStyle Lite™, Abbott Diabetes Care Inc., USA), rats with blood glucose over 300 mg/dL were considered as diabetics. An oral daily 0.5% CMC was given to normal and diabetic control rats. *Sita* treated group received *Sita* (10 mg/kg) orally for 7 weeks starting from day 1 of confirmation of diabetes. Blood samples were collected from tail vein weekly for 7 weeks. Animals were sacrificed at the end of week 7, pancreases were dissected and each was cut into 2 parts. The 1st part was for immunohistochemical and histopathological examination and the 2nd part as homogenate for assessment of biochemical parameters.

Assessment of blood biochemical parameters: Plasma glucose was measures using a glucometer instrument (FreeStyle Lite™, Abbott Diabetes Care Inc., USA). Glycated hemoglobin (HbA_{1c}) was determined by HbA_{1c} kit (Cat. No. 80300, Crystal Chem INC, Spain). Serum insulin was determined by ELISA Kit for insulin (Cat. No. CEA448Ra, Cloud-Clone Corp., USA); Serum Glucagon was measured using glucagon ELISA Kit (Cat. No. EIAR-GLU, RayBio®, USA); All following manufacturer's instructions.

Assessment of pancreatic Nitrite/Nitrate (NO) concentration, Malondialdehyde (MDA) level, reduced glutathione (GSH) concentration and superoxide dismutase (SOD) activity in pancreatic homogenate: Nitrite/nitrate ($NO2^-$/$NO3^-$) concentration was measured as stated by Green et al. [10]. Pancreatic MDA content was measured using the technique described by Kei [11]. Concentration of GSH was determined following the method designated by Beutler et al. [12], and SOD activity was estimated using to the technique described by Nishikimi et al. [13]. All using assay kits commercially available from Bio Diagnostic (Giza, Egypt). Tests' procedures were carried out according to supplied manufacturer instructions.

Histopathological examination of pancreatic tissue and immunohistochemical analysis: Pancreatic specimens were fixed in 10% v/v neutral buffered formalin for 48 hours. Then paraffin blocks of specimens were prepared using standard histopathological techniques. A microtome (Leica RM 2125RT, Germany) was used to cut paraffin sections (5 µm) serially. These sections were then mounted on glass slides, and stained with hematoxylin-eosin (H&E) for histopathological examination [14], and anti-insulin stain for immunohistochemical analysis. A digital camera installed on Olympus® microscope was used to take photos. Then these photos were analyzed using VideoTest® Morphology® software (Russia) with a specific built-in routine for geometrical description analysis, area measurement and stain density determination.

Statistical analysis: The results are presented as mean ± standard error of mean (mean ± SEM). They were analyzed using GraphPad Prism (version 6.01) and GraphPad InStat (version 3.01). Regression analyses were used for construction of standard curves.

RESULTS

Effect of Sita on STZ-induced mortality in rats: Streptozotocin significantly increased percentage of mortality in diabetic control group by 50 % when compared to normal group (Table1). While oral treatment with Sita for 7 weeks decreased percentage of mortality to 10 %.

Effect of Sita on fasting blood glucose levels in STZ-induced diabetic rats: Blood glucose levels (BGL) (Table2) increased in all groups injected by STZ when compared to normal "non-injected" group. Oral administration of *Sita* for 7 weeks significantly decreased plasma glucose levels after 1, 2, 3, 4, 5, 6 & 7 weeks when compared to the corresponding levels of the diabetic control group. Also there were 37.1, 51.5, 56.5, 64.5, 65.6, 69 & 69.7% decrease in plasma glucose level after 1, 2, 3, 4, 5, 6 & 7 weeks of treatment, respectively, in comparison to their corresponding initial value.

Effect of Sita on glycated hemoglobin (HbA_{1c}) in STZ-induced diabetic rats: At the end of week 7, diabetic control group revealed a significant increase in HbA_{1c} by 96.2% when compared to normal group (Figure 1). There was a 51.6% decrease in HbA_{1c} in *Sita* treated animals when compared with diabetic control one (P<0.05).

Effect of Sita on serum insulin levels in STZ-induced diabetic rats: Single intra-peritoneal injection of STZ showed a significant increase in serum insulin level by 50.14 % in diabetic control when compared to normal group (Figure 2). In Sita treated group, there was an increase in serum insulin level by 25.42% but this difference considered non-significant in comparison to diabetic control group while there was a significant difference when compared to normal control group.

Effect of Sita on serum glucagon levels in STZ-induced diabetic rats: There was a significant increase in serum glucagon level by 397% when compared to normal group (Figure 3). Treatment with Sita significantly decrease serum glucagon level by 45.67% when compared to the diabetic control one; also there was a significant difference in comparison with normal group.

Effect of Sita on MDA, GSH, SOD and NO levels in pancreatic homogenate in STZ-induced diabetic rats: Effect of oral administration of Sita once daily for 7 weeks on MDA, GSH, SOD and NO levels in pancreatic homogenate in diabetic rats are illustrated in Figure 4, 5, 6 and 7 respectively. Diabetic control injected by STZ showed a significant increase in MDA and NO levels by 30% and 600% respectively. Also there was a significant decrease in GSH content and SOD activity by 30.38% and 78.1% respectively, when compared to normal group (P<0.05). *Sita* significantly decreased MDA and NO levels by 16.75 % and 39.8% respectively; While it significantly increased GSH content and SOD activity by 33.09% and 146.96% respectively, when compared with diabetic control group (P<0.05).

Effect of Sita on histopathological examination of isolated pancreatic tissues in STZ-induced diabetic rats: Histopathological examination of pancreatic tissues stained with (H&E), showed normal islets of Langerhans in between normal pancreatic acini in normal control group (Figure 8A); while in diabetic control group, there was an obvious destruction of islets of Langerhans with obscure margins, degeneration of the islets with irregular intercellular spaces and cells with pyknotic nuclei and vacuolar changes (Figure 8B). In Sita treated group, islets of Langerhans showing pyknotic among normal ones with the presence of hyaline material in intercellular spaces (Figure 8C). In immunohistochemical analysis using anti-insulin antibody, normal control group revealed a positively stained beta cells in islets of Langerhans (Figure 9A); while in diabetic group, low insulin immune-reactivity was observed (Figure 9B). Treatment with *Sita* showed a positive immune-reactivity (Figure 9C). The statistical analysis indicated that the percentage of stained area was very high in normal control and significantly low in diabetic control. *Sita* treated group revealed 38.63% increase in percentage of area stained when compared to diabetic control group but this difference considered non-significant (Figure 10).

DISCUSSION

Patients with T1D need a lifelong insulin therapy. Most of them need two or more injections of insulin daily, with doses based on measurement of glucose levels to avoid diabetes related acute and late complications. The aim of insulin therapy in T1D is to be similar to insulin secretion of a normally functioning pancreas. Current treatments still do not resemble the pancreatic β-cells' endogenous insulin profile, and all still show risks of hypoglycemia, ketosis and suboptimal control [3].

Currently, DPP-4 inhibitors are approved for management of T2D but not for T1D. DPP-4 inhibitors enhance the actions of both GLP-1 and GIP and can be taken orally. In addition, GLP-1 elicits a glucose dependent insulin release [15]; therefore, DPP-4 inhibitors have lower risk to induce hypoglycemia. In T2D patients, administration of DPP-4 inhibitors improved β-cell function including Homeostasis Model Assessment–β (HOMA-β), proinsulin to insulin ratio and parameters of beta cell responsiveness in meal tolerance test. They also improved alpha cells' sensitivity to glucose, causing a more glucose dependent glucagon secretion [7].

Recently, although most patients with chronic T1D have few β-cells, there are claims that evidences for

β-cell regeneration in very young children and infants are present, but not in adults [16].

In this study, the potential role of *Sita* (DPP-4 inhibitors) as oral treatment regimen for **T1D** was assessed using STZ-induced T1D model in rats.
Administration of a single intra-peritoneal dose of STZ (65mg/kg) induced T1D [9]. Then, monitoring of fasting blood glucose level (BGL) weekly for 7 weeks. At the end of week 7, HbA_{1c}, insulin, glucagon, MDA, NO, GSH and SOD were measured. Also, histopathological and immunohistochemical examination of pancreatic tissues were performed using (H&E) and anti-insulin antibody respectively. Finally percentage of mortality was calculated for each group.

Regarding BGL, oral administration of *Sita* in diabetic rats significantly decreased BGL when compared to the corresponding initial value as well as to the equivalent weekly values of the diabetic control group. It decreased blood glucose levels by 37.1, 51.5, 56.5, 64.5, 65.6, 69 & 69.7 after 1, 2, 3, 4, 5, 6 & 7 weeks of treatment respectively, when compared to their corresponding initial value. While when compared to the corresponding weekly diabetic control group values, there were 41.3, 53.8, 55.6, 62.3, 63.8, 68 & 68.9% decrease in blood glucose level after 1, 2, 3, 4, 5, 6 & 7 weeks of treatment, respectively.

Glycated hemoglobin (HbA_{1c}) is currently recommended for testing and monitoring diabetes. Koenig *et al*. first suggested the use of HbA_{1c} as a biomarker for monitoring BGL among diabetic patients [17]. Measuring HbA_{1c} offers indication about an individual's average BGL during the previous 2 to 3 months [18]. The prognostic potential of HbA_{1c} lies in its distinctive capability of evaluating retrospective glycemic control.

In *Sita* treated group, there was statistically significant decrease in HbA_{1c} by 51.6% when compared to the diabetic control group at level of significance (P<0.05). HbA_{1c} values of these treated groups declined to approximately reach normal values, with statistically non-significant difference when compared to the normal control.

These outcomes (BGL & HbA_{1c}) propose that *Sita* may have a potential anti-hyperglycemic activity in T1D besides their approved role in T2D; but the mechanisms of such activity remain unclear. From the pathophysiology of T1D, it is assumed to be caused by destruction of beta cells. There are some claims that beta cells are not totally destroyed but only become non-functional [19]. The net result is that there is a decrease in insulin level. However, there is an increasing appreciation of defects in other gluco-regulatory cells in T1D such as over secretion of glucagon from pancreatic alpha cells [20]. Thus the anti-hyperglycemic effect of *Sita* observed here may be due to either decreasing serum glucagon level or increasing serum insulin levels through preserving remained beta cell, activation of non-functional beta cells or beta cells' regeneration. Also, extra pancreatic mechanisms may be involved.

In comparison to the diabetic control group, *Sita* treated groups showed an increase in serum insulin level by 25.42%. But this increase considered statistically non-significant. Pospisilik et al. reported marked enhanced insulin secretory capacity upon treatment with P32/98, an experimental DPP-4 inhibitor [21]. Our work implies an approved DPP-4 inhibitors for T2D that clearly showed consistent results with that of Pospisilik et al.

The paracrine/endocrine inhibitory influence of insulin on glucagon secretion have been recognized. There is a reciprocal relationship between insulin and glucagon in maintaining glucose homeostasis. Improperly increased alpha-cell function significantly contributes to hyperglycemia and reflects the loss of limitation normally exerted by high levels of insulin on alpha-cells [22].

Interestingly, *Sita* showed a significant decrease in serum glucagon levels by 45.67% when compared to diabetic control (P<0.05). *Sita* can control the inappropriate glucagon secretion which in turn deceases the hepatic glucose output and thus reduces overall hyperglycemia in T1D as just as its recognized role in T2D. These findings are consistent with Ahren et al. whom reported that inhibition of DPP-4 in a clinical trial reduced glycaemia, sustained insulin levels, and reduced glucagon levels even though their study was designed on T2D [24].

Nerup et al. reported that, damage of β-cells through autoimmune attack and subsequent development of T1D involves reactive oxygen species (ROS) and NO that might be generated by the β-cells themselves upon stimulation by TNF and IL-1 [25]. Certainly, β-cells are extremely prone to oxidative destruction [26].

ROS generate the lipid peroxidation progression in an organism. MDA is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. MDA level is commonly known as a marker of oxidative stress and the antioxidant status [27]. On measuring MDA levels

in pancreatic tissue homogenates, diabetic control group showed increased level by 30 % more than normal group. In *Sita* treated groups this percent decreased to 8.5% in comparison with normal group.

Tissue NO levels increased by 600% in diabetic control group compared to normal. While in *Sita* treated group there was 39.8% decrease when compared to diabetic control. This treatment regimen significantly decreased NO levels and so decreased oxidative stress.

Along with direct effects of ROS, oxidative stress might be caused by an imbalance between anti-oxidative enzymes (GSH and SOD) and the activity of endogenous pro-oxidative enzymes (such as mitochondrial respiratory chain enzymes and NADPH oxidase). The pro-oxidative enzymes outweighs the balance [28]. Upon measuring GSH content and SOD activity, the most important anti-oxidative enzymes, diabetic control showed a significant decrease in GSH level and SOD activity by 30% and 78% respectively, when compared to normal group. Oral administration of *Sita* significantly increased GSH content and SOD activity by 33% and 147% respectively, when compared to diabetic control group.

These results suggest that *Sita* have a significant anti-oxidant and free radical scavenging activities. This may be due to direct anti-oxidant activity of DPP-4 inhibitors as reported by Maeda et al. [29], or may be due to blunting daily acute glucose fluctuations as reported by Rizzo et al. [30]. Butler et al. reported that in patients with chronic diabetes, the pancreas lacks insulin-producing cells and the residual β-cells are unable of regeneration [31]; And due to enhancement of nearly all measured diabetic biochemical markers, histopathological and immunohistochemical examination of pancreatic tissues were performed as an attempt to assess the involved mechanisms of action.

Diabetic control showed an obvious destruction of islets of Langerhans with obscure margins, degeneration of the islets with irregular intercellular spaces and cells with pyknotic nuclei and vacuolar changes was found using (H&E); while in *Sita* treated group, islets of Langerhans showing pyknotic among normal ones with the presence of hyaline material in intercellular spaces. The percentage of area stained with anti-insulin antibody increased in *Sita* group by 38.63% when compared with the diabetic one although this increase was non-significant. This indicates that *Sita* may have a mild effect in induction of β-cells regeneration.

CONCLUSION

Sita has mild ability to induce β-cell regeneration and/or to activate non-functioning cells which in-turn result in an increase in insulin secretion. This effect may arise from its direct action on β-cells and/or preserving the already functioning β-cells. In addition to its action on β-cells, *Sita* also has an effect on decreasing the inappropriate glucagon secretions. It also has strong anti-oxidant activity. All these actions give rise to improvement in overall glycaemia control which was obvious in blood glucose levels and glycated hemoglobin. Thus *Sita* can be considered as an oral treatment regimen for T1D. Further clinical studies are recommended before clinical application.

Table 1: Effect of *Sita* on STZ-induced mortality.

Treatment	**Mortality (%)**
Normal control	0
Diabetic control	50 #
Sita	10 *

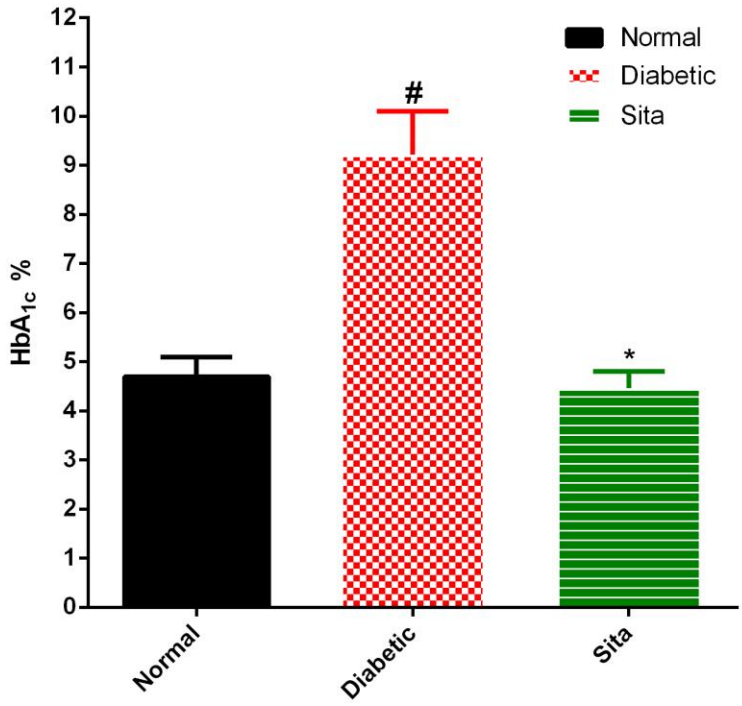
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
(#), (*) significantly different from normal control and diabetic control respectively, using Kruskal-Wallis test followed by Dunn's post hoc test at ($p< 0.05$).

Table 2: Effect of *Sita* on plasma glucose level in STZ-induced diabetic rats throughout seven weeks.

	Plasma glucose level, mg/dl		
	Normal control	**Diabetic control**	***Sita***
Initial	91 ±7	450 ±18 #	439 ±15 #
Week 1	85 ±5	470 ±16 #	276 ±15 #*†
Week 2	89 ±7	461 ±12 #	213 ±22 #*†
Week 3	84 ±4	430 ±25 #	191 ±20 #*†
Week 4	90 ±4	414 ±18 #	156 ±16 *†
Week 5	79 ±5	417 ±30 #	151 ±15 *†
Week 6	86 ±4	425 ±22 #	136 ±13 *†
Week 7	90 ±8	428 ±30 #	133 ±10 *†

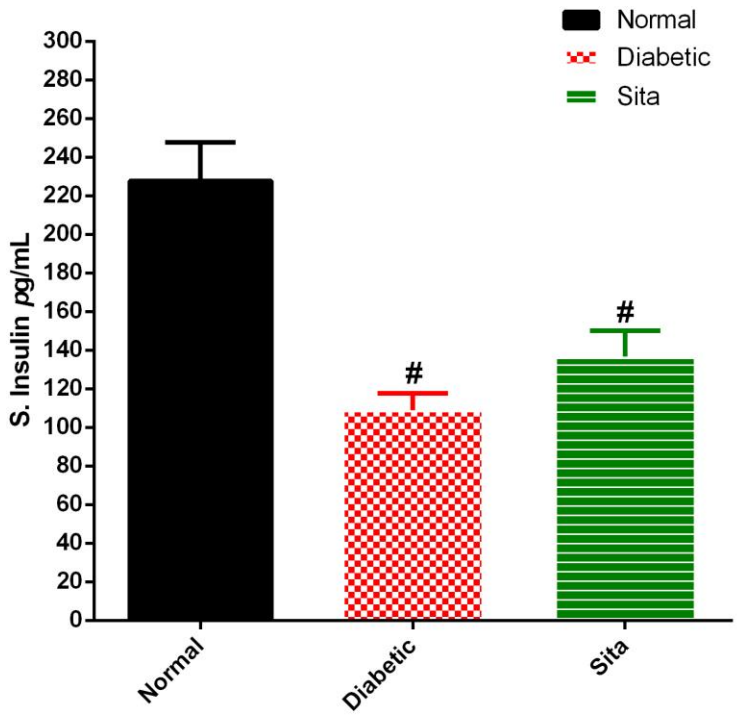
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Values represent the mean ± SEM, n=10.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).
(†) Significantly different from Sitagliptin initial value using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).

Figure 1: Effect of *Sita* on glycated hemoglobin (HbA_{1c}) values in STZ-induced diabetic rats.



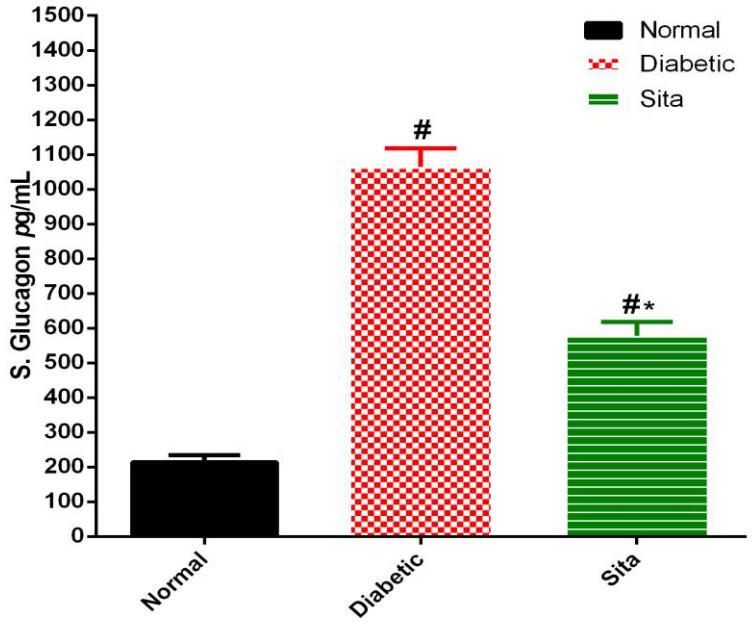
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of HbA1c were done at the end of experiment "end of week 7"
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).

Figure 2: Effect of *Sita* on serum insulin levels in STZ-induced diabetic rats.



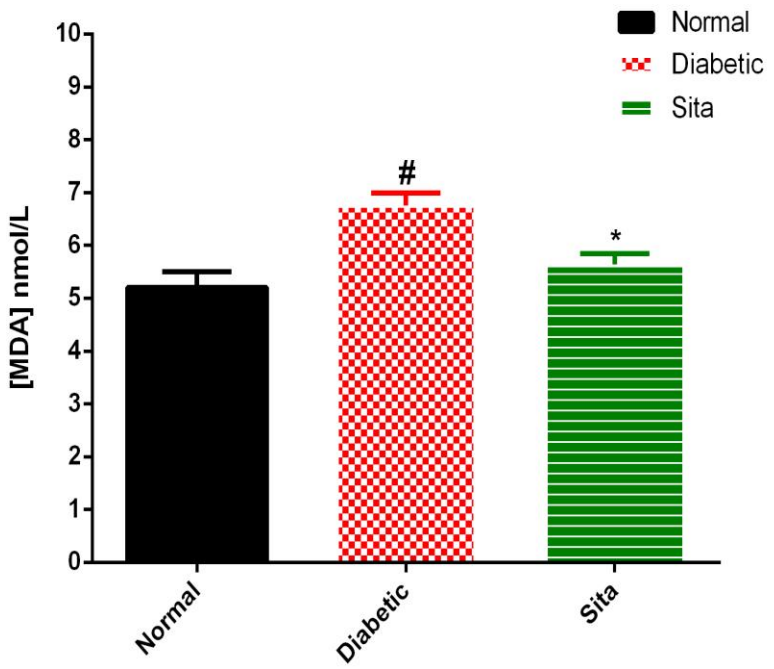
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of serum insulin levels were done at the end of experiment "end of week 7"
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).

Figure 3: Effect of *Sita* on serum glucagon levels in STZ-induced diabetic rats.



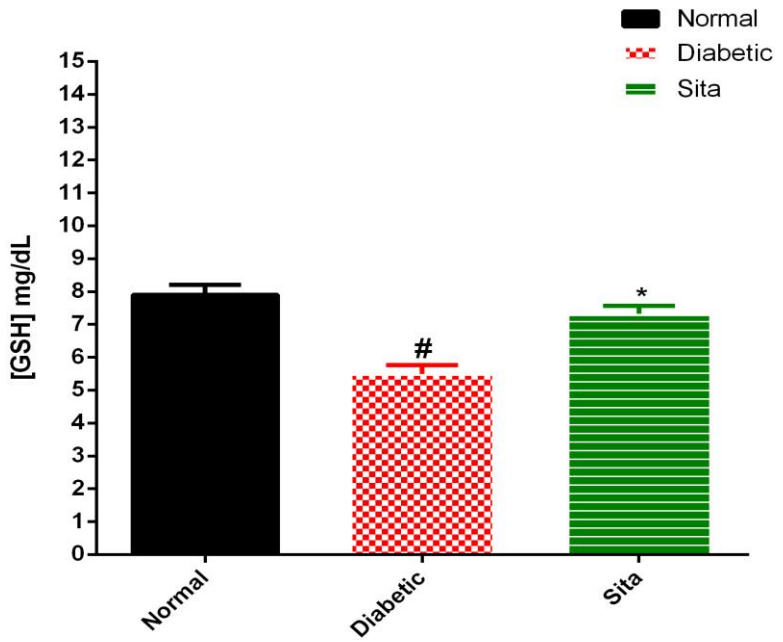
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of serum glucagon levels were done at the end of experiment "end of week 7"
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).

Figure 4: Effect of *Sita* on pancreatic malonaldhyde (MDA) levels in STZ-induced diabetic rats.



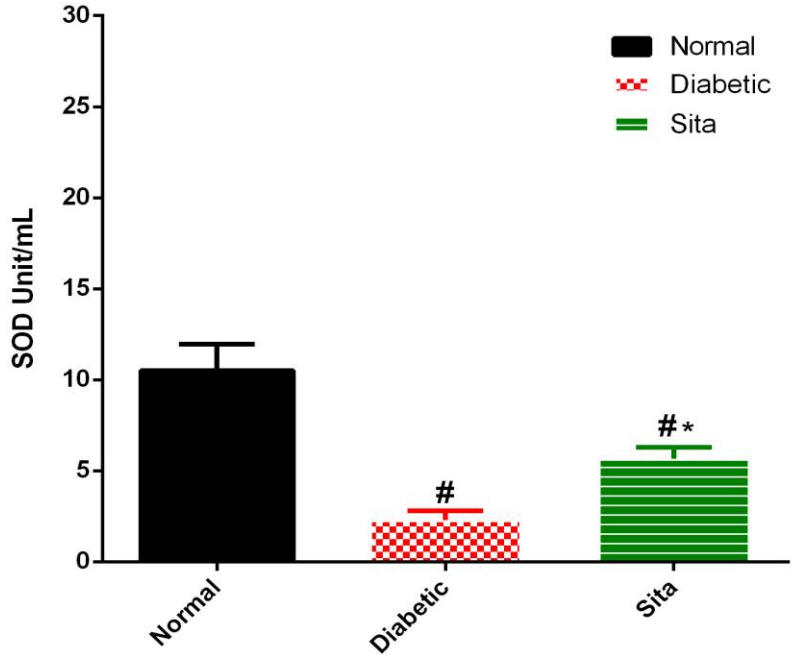
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of MDA levels were performed at the end of the experiment (week 7).
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p < 0.05$).

Figure 5: Effect of Sita on pancreatic glutathione (GSH) levels in STZ-induced diabetic rats.



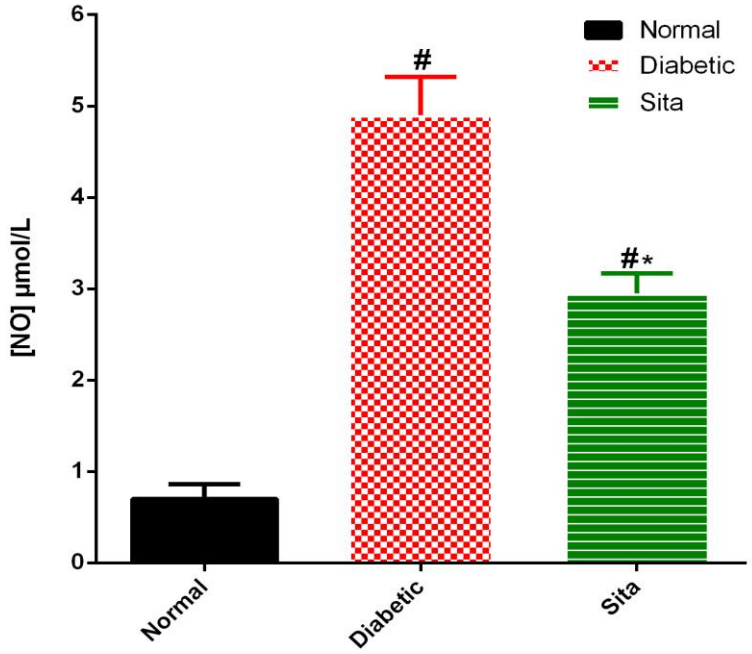
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of GSH levels were performed at the end of the experiment (week 7).
Values represent the mean ± SEM
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p < 0.05$).

Figure 6: Effect of Sita on pancreatic superoxide dismutase activity (SOD) in STZ-induced diabetic rats.



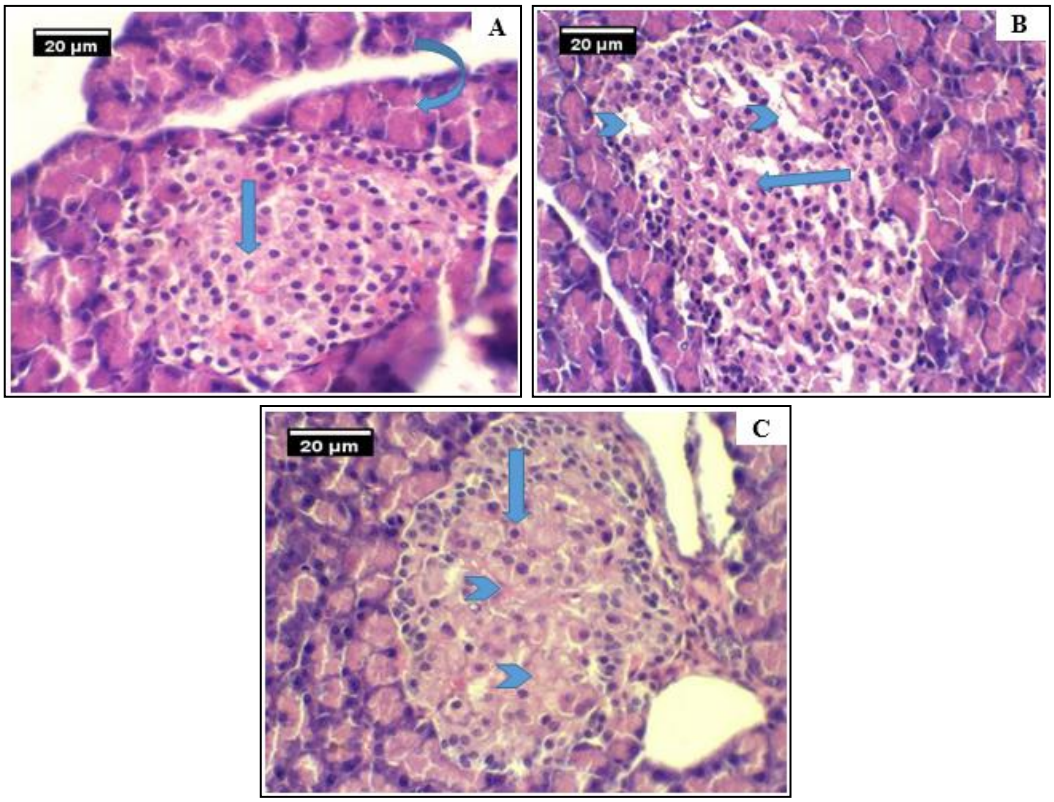
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of SOD activity were performed at the end of the experiment (week 7).
Values represent the mean ± SEM; (#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$).

Figure 7: Effect of *Sita* on pancreatic Nitrite/Nitrate (NO) levels in STZ-induced diabetic rats.



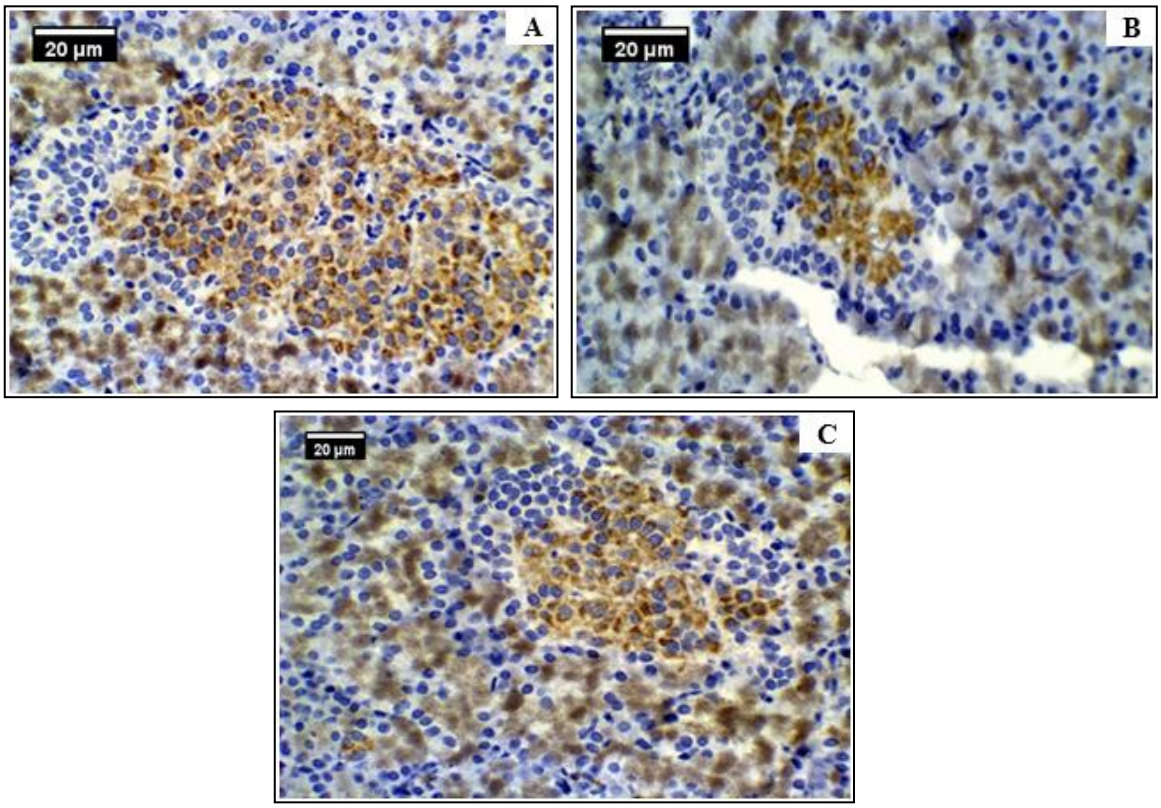
Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of NO levels were performed at the end of the experiment (week 7).
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p< 0.05$)

Figure 8: Photomicrographs of stained pancreatic sections using (H&E) (x400).



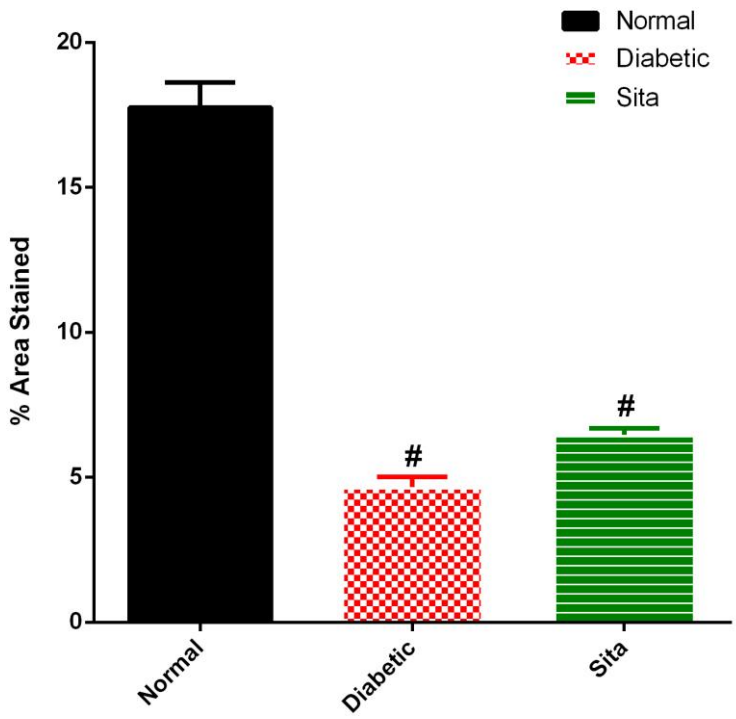
(A) Normal control group, showing normal islets of Langerhans (arrow) in between normal pancreatic acini (curved arrow). (B) Diabetic control group, pancreas showing destructed islets of Langerhans with obscure margins and cells with pyknotic nuclei and vacuolar changes (arrow), degeneration of the islets with irregular intercellular spaces (arrow head). (C) Sita treated group, islets of Langerhans showing pyknotic (arrow) among normal ones with the presence of hyaline material in intercellular spaces (arrow head).

Figure 9: Photomicrographs of stained pancreatic sections using immuno-histochemichal anti-insulin anti-body.



(A) Normal Control group, showing significant positively stained β-cells in an islet of Langerhans. (B) Diabetic group, low insulin immune-reactivity was observed in the islet cells. (C) Sita treated group, islets of Langerhans with positive immunoreactivity.

Figure 10: Effect of *Sita* on percentage of area stained of pancreatic sections using anti-insulin anti-body in STZ-induced diabetic rats.



Sita (Sitagliptin 10 mg/kg) was given once daily orally for 7 weeks.
Measurements of percentage of area stained were performed at the end of the experiment (week 7).
Values represent the mean ± SEM.
(#), (*) significantly different from normal control and diabetic control respectively, using one-way ANOVA followed by Tukey Kramer multiple comparisons test at ($p < 0.05$).

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