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Role of Vildagliptin against Destruction of Pancreatic Beta-Cells in Type 2 Diabetes

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Abstract: Type 2 diabetes mellitus is a growing global public health problem caused by a combination of pancreatic beta-cells dysfunction and insulin resistance. Vildagliptin is one of the Dipeptidyl peptidase-4 (DPP-4) inhibitors that appear to improve insulin secretion and insulin sensitivity. This study aimed to explore the role of vildagliptin against the destruction of pancreatic beta-cells. Vildagliptin was orally administrated to both normal and diabetic Wistar rats for 4 weeks, followed by an investigation of biochemical, flow cytometrical, and morphological analysis of pancreatic islets. After 4 weeks of treatment, vildagliptin increased plasma insulin and active glucagon-like peptide-1 (GLP-1) levels and decreased blood glucose and glucagon levels; also inhibited oxidative stress by reducing Malondialdehyde (MDA) levels and increasing both Superoxide dismutase (SOD) and catalase (CAT) activities, also inhibited pancreatic islets apoptosis by increasing percentages of viability and decreasing percentages of necrosis, early apoptosis and late apoptosis of diabetic rats islets in compared to untreated diabetic rats. Further, as shown in an immunohistochemical examination, vildagliptin enhanced pancreatic beta-cell proliferation by increasing insulin expression within the pancreatic islets. To conclude, vildagliptin promoted pancreatic beta-cells survival in diabetic rats by improving overall glycemic control and alleviating pancreatic beta-cell apoptosis and oxidative stress.

Keywords: Type 2 Diabetes; Pancreatic beta-cells; Vildagliptin; Insulin.

1. Introduction

Diabetes mellitus is one of the most growing public health worldwide, 90% of diabetic patients have type 2 diabetes [1]. Type 2 diabetes could be associated with many complications and affect multiple organs that may cause a huge burden to society [2]. It is a common metabolic disorder that is caused by a combination of pancreatic beta-cell dysfunction that leads to insulin secretion deficiency and insulin resistance. Defects in any of the mechanisms involved in these processes can cause a metabolic imbalance responsible for the disease's development [3]. Beta-cell failure is a key to the onset and progression of type 2 diabetes because of impaired function and reduced mass [4]. Pancreatic regeneration is a potential therapeutic strategy for the recovery of beta-cell loss. Almost all diabetic drugs can protect pancreatic beta-cells by inhibiting beta-cell apoptosis and dedifferentiation by correcting hyperglycemia and improving the consequent inflammation and oxidative stress. Several glucose-lowering agents, including glucagon-like peptide-1 have been shown to promote beta-cell proliferation, considered the main source of the regeneration of pancreatic beta-cells in adult rodents [5].

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are known as the incretin hormones, are released from enter-endocrine cells in response to nutrient presence in the small intestines [6]. Incretin hormones facilitate glucose regulation by stimulating glucose-dependent insulin secretion and suppressing glucagon secretion. Type 2 diabetes patients have impaired insulin response to incretin hormones (GLP-1, GIP), contributing to hyperglycemia [7]. Dipeptidyl peptidase-4 (DPP-4) is a proteolytic enzyme that is responsible for the rapid degradation of GLP-1, which is required for the secretion of insulin [8], so much research has been focused on the development of incretin-based type 2 diabetes therapies that included incretin receptor agonists and dipeptidyl peptidase-4 enzyme inhibitors [9]. Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a class of oral glucose-lowering agents that inhibit DPP-4 enzyme, thus blocking the breakdown of GLP-1 and GIP to increase levels of the active hormones and restoring many of the pathophysiological problems of diabetes [10], so Vildagliptin as a Dpp-4 inhibitor is used in the management of diabetes by providing anti-hyperglycemic effects [11] through several molecular mechanisms including promoting insulin secretion, suppression of glucagon secretion and reduction of fatty acid flux from the adipocyte. Vildagliptin is also associated with improving beta-cell function, which is likely secondary to the improved metabolic state. Despite there being no evidence of restoration of beta-cell mass [12,13]. Oxidative stress induced by hyperglycemia plays an important role in pancreatic beta-cell dysfunction and apoptosis, as well as in the development and progression of diabetic complications, so the antioxidant effect reported to vildagliptin may protect pancreatic beta-cells from damage[14].

In this study, we aimed to explore the role of vildagliptin against the destruction of the pancreatic beta-cells, whether by inhibiting pancreatic beta-cell apoptosis or decreasing oxidative stress in type 2 diabetic rats.

2. Materials and Methods

2.1. Animals and experimental protocol.

Thirty-two Wistar male rats, 8 to 10 weeks old, weighed about 150 to 200 g were used. At least 5 days prior to the start of the experiment, housed three to five male rats per cage at 24°C±1°C and 55%±5% humidity, with a 12-hour light-dark cycle (light on at 8:00 and off at 20:00). Rats were provided water and laboratory chow diet during the whole experiment period. All animals received well catered following the Animal Care and Use guidelines in accordance with the National Institutes of Health “guide for the care and use of laboratory animals” (NIH Publications No.8023, revised 1978). Approval was granted by the Ethics Committee of Mansoura University (No SC.MS.23.02.17).”

After the adoption period, rats were randomly divided into four groups, and each group included eight rats:

I. Normal control group. Rats received no treatment.

II. Normal rats treated with the vildagliptin group. Rats received vildagliptin (50 mg/kg body weight/day, oral) as an emulsion in distilled water for 4 weeks.

III. Diabetic control group. Type 2 diabetes was induced by the administration of nicotinamide (230mg/kg) to afford partial protection of beta-cells against streptozotocin (65mg/kg); after 10 days of injection, the blood glucose level from a tail vein was tested to ensure induction of type2 diabetes as the blood glucose concentrations should be >150 mg/dl to ensure hyperglycemia [15].

IV. Diabetic rats treated with vildagliptin group. Type 2 diabetes was induced as group III and received vildagliptin (50 mg/kg body weight/day, oral) as an emulsion in distilled water for 4 weeks.

2.2. Blood and sample collection.

At the end of the experiment, rats were sacrificed using an approved method of euthanasia; whole blood samples were collected from all rats under investigation via cardiac puncture. The blood was divided into two parts; one part was delivered to plain tubes and allowed to clot for 10-15 minutes, centrifuged, and serum was separated and stored in small aliquots at -20°C.The other part of the whole blood was taken in EDTA-coated tubes to keep it liquid for further biochemical analysis.

Pancreatic tissues were quickly removed and rinsed with sterile cold isotonic (0.9% NaCl). The pancreatic tissue samples were divided into two parts. One part was stored in clean tubes containing 5 ml of neutral formaldehyde solution (10%) for histopathological and immunohistochemical examination. The other part is stored at -20˚C for cell cycle analysis.

2.3. Biochemical analysis and antioxidant assays.

The level of glucose was measured colorimetrically according to the procedures of [16]. The insulin level was evaluated according to the quantitative sandwich enzyme immunoassay technique by using rat ELISA kits for insulin (Cusabio Biotech, Wuhan, China). The level of glucagon was measured according to the quantitative sandwich enzyme immunoassay technique by using rat ELISA kits for rat glucagon (Cusabio Biotech, Wuhan, China). The level of glucagon-like peptide-1 GLP-1 was evaluated according to the quantitative sandwich enzyme immunoassay technique using rat ELISA kits for rat GLP-1 (Cusabio Biotech, Wuhan, China). The level of MDA was evaluated photometrically according to the procedures of [17]. The enzymatic activity of SOD was estimated according to the procedures of [18]. The enzymatic activity of catalase was estimated according to the procedures of [19].

2.4. Cell cycle analysis.

The percentage of viability, necrosis, early apoptosis, and late apoptosis in pancreatic islets were evaluated by flow cytometric analysis according to the method of [20] by using the double staining annexin-v and propidium iodide method.

2.5. Histopathology and immunohistochemistry.

The pancreatic tissues were histopathologically examined using hematoxylin (H) and eosin (E) staining routines, examined under bright field light microscopy, and photographed according to the method of [21]. The anti-insulin antibody in pancreatic tissues was immunohistochemically evaluated according to the method of [22].

2.6. Statistical analysis.

For all statistical analysis, the Graph Pad Prism package and SPSS software (SPSS Inc., Chicago, IL) were used. The results were expressed as mean ± (SE) [23]. P value ≤ 0.05 was considered the minimal level of significance.

3. Results and Discussion

3.1. Results.

3.1.1. The effect of vildagliptin on blood glucose.

According to results arranged in Figure (1A), rats in the diabetic group showed significantly higher levels of blood glucose in comparison to the normal group, while the vildagliptin-treated group showed a significant reduction in blood glucose in contrast to the diabetic group.

3.1.2. The effect of vildagliptin on plasma insulin, glucagon, and active GLP-1.

As illustrated in Figure 1 (b), the diabetic group showed a significant reduction of plasma insulin relative to the normal group, while the vildagliptin-treated group showed a significant increase compared to the diabetic group. Also, according to results in Figure 1 (C), rats in the diabetic group showed significantly higher levels of glucagon in comparison to the normal group, while the vildagliptin-treated group showed a significant reduction in glucagon in comparison to the diabetic group. Furthermore, as shown in Figure 1 (D), levels of plasma active GLP-1 were not significantly lower in the person with diabetes compared to the normal group, while vildagliptin treatment significantly augmented plasma active GLP-1 compared to the diabetic group.

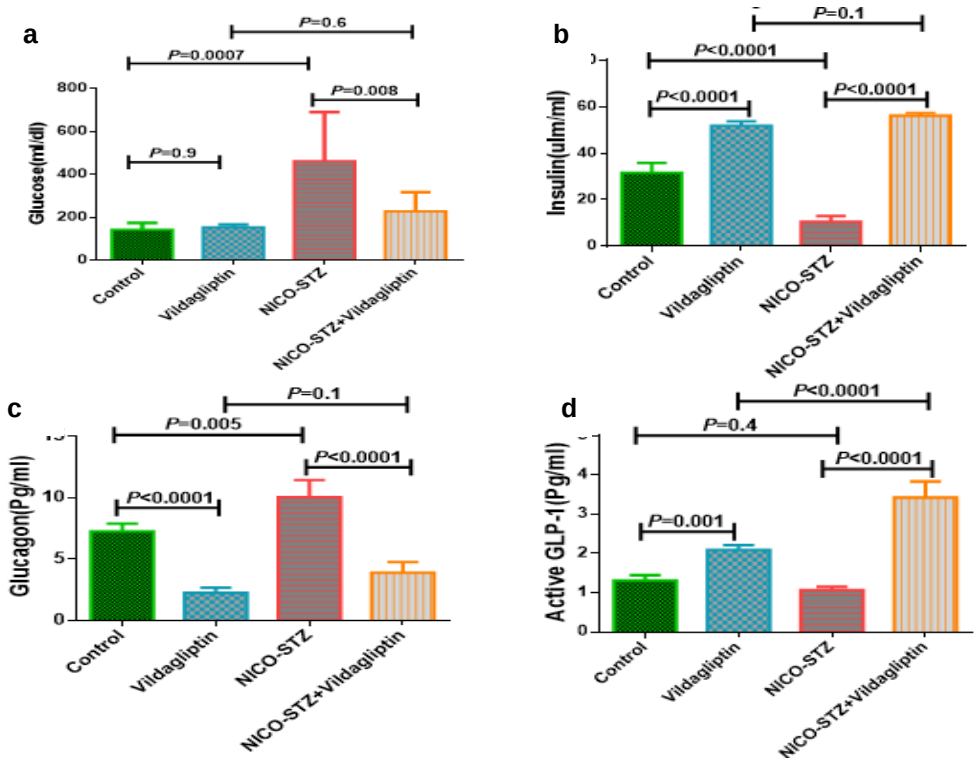

Figure 1. (a) Changes in blood glucose, **(b)** plasma insulin, **(c)** plasma glucagon, **(d)** plasma active GLP-1 in different groups compared to the control.

3.1.3. The effect of vildagliptin on Malondialdehyde (MDA) level, Superoxide dismutase (SOD), and catalase activity.

Results arranged in Figure 2 (a) showed that levels of Serum MDA were significantly increased in diabetic rats compared to a control group. At the same time, the treatment with vildagliptin significantly reduced MDA levels. Results arranged in both Figure 2 (b) and Figure

2 (c) respectively showed that the activities of both SOD and catalase were significantly decreased in diabetic rats than normal group. At the same time, vildagliptin treatment significantly increased both SOD and catalase activities.

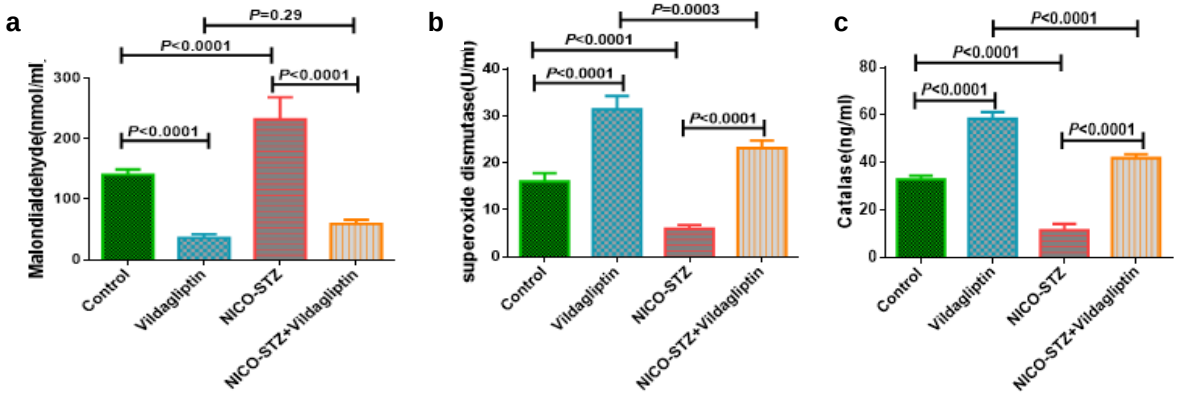

Figure 2. Changes in **(a)** Malondialdehyde level, **(b)** Superoxide dismutase activity, **(c)** Catalase activity in treated groups compared to the control.

3.1.4. Cell cycle progression and apoptosis in pancreatic tissue of different treated groups and the control.

The results of analysis of flow cytometry quadrant histogram figure 3 showing double stain of annexin-v and propidium iodide indicated percentages of viability were very significantly decreased in diabetic rats when compared with control figure 4 (a), while percentages of necrosis, early apoptosis, and late apoptosis were significantly increased figure 4 (b-d). The results also indicated that the treatment with vildagliptin significantly increased viability percentages and decreased both necrosis and late apoptosis but not significantly decreased percentages of early apoptosis.

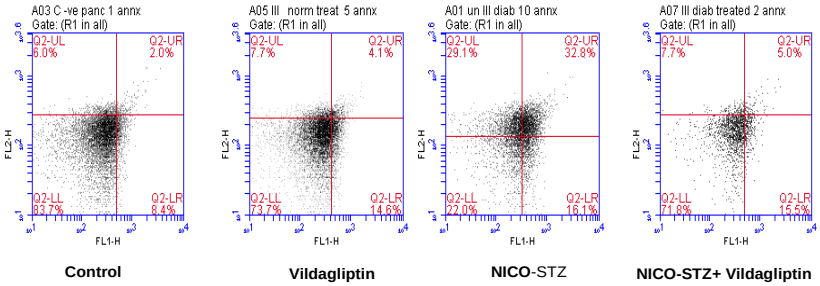

Figure 3. Flow cytometry quadrant histogram showing double stain of annexin-v and propidium iodide indicating the effect of vidagliptin (50mg/kg) on a percentage of **(a)** viability, **(b)** necrosis, **(c)** early apoptosis, **(d)** late apoptosis.

3.1.5. Morphological analysis of pancreatic islets.

Morphological examination confirmed and strengthened the results obtained by cell cycle analysis. Histopathological sections of the pancreatic islet showed that treatment with vildagliptin decreased the apoptosis to mild or moderate degenerative lesions associated with macrophages infiltration in diabetic rats, as shown in Figure 4 (d) compared to untreated diabetic rats in figure 4 (c) that showed necrosis of pancreatic islets, infiltration with macrophages and apoptosis within the pancreatic acini. Also, immunohistochemical sections of the pancreatic islet showed that treatment with vildagliptin resulted in a marked increase of insulin expression within the pancreatic islets of Langerhans and anti-insulin antibodies in

diabetic rats, as shown in Figure 5 (D) compared to untreated rats as figure 5 (c) which showed marked decrease of immunostaining of insulin within the pancreatic islets of Langerhans.

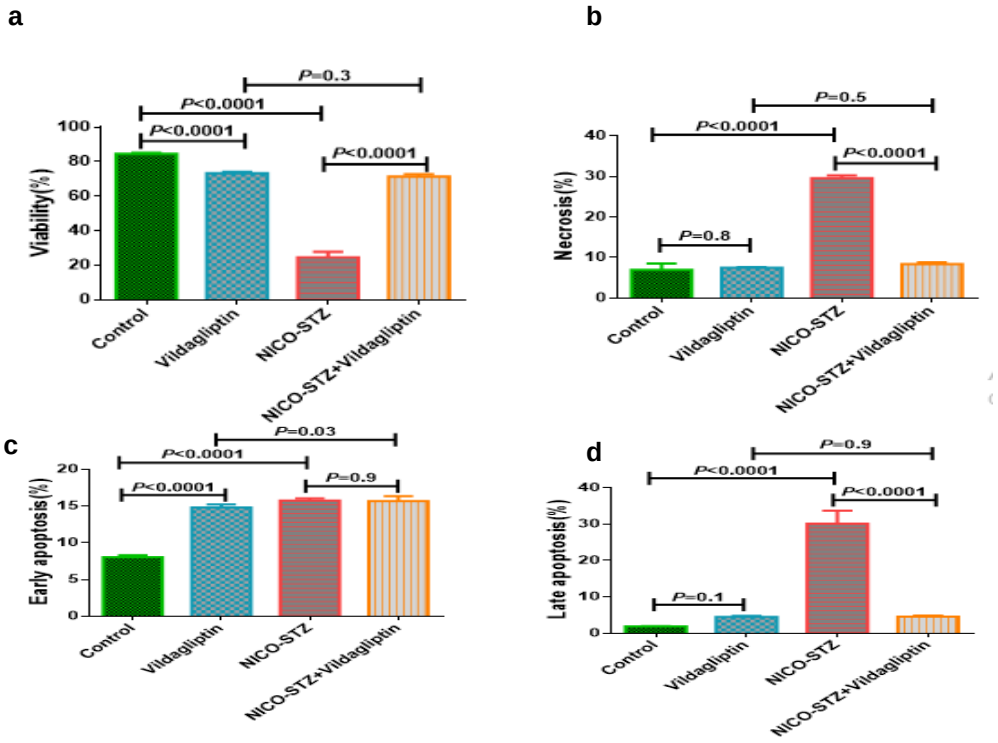

Figure 4. The effect of vildagliptin (50mg/kg) on a percentage of (a) viability, (b), necrosis, (c) early apoptosis, (d) late apoptosis of pancreatic beta cells in normal and diabetic rats.

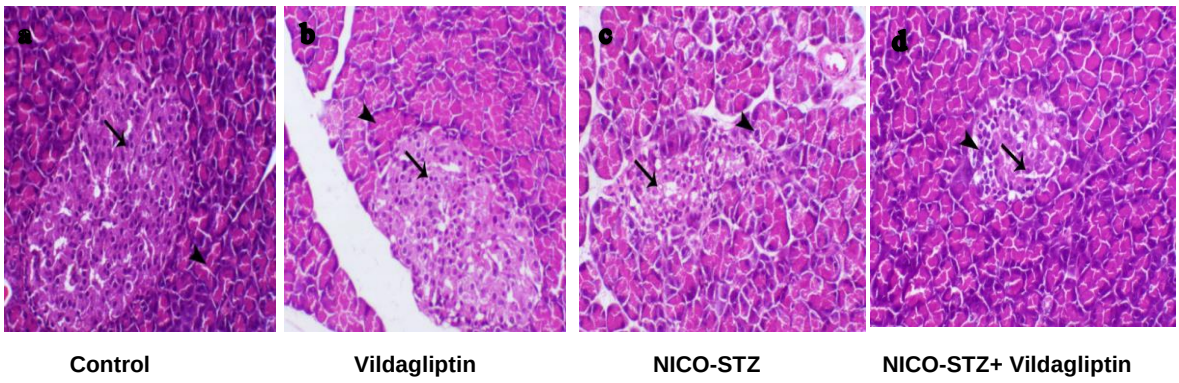

Figure 5. Effect of vildagliptin on histopathological images of (a) normal control rats, (b) normal rats treated with vildagliptin, (c) diabetic rats, (d) diabetic rats treated with vildagliptin, stained with H&E.

3.2. Discussion

Diabetes is one of the most common chronic diseases in the world that is characterized by pancreatic beta-cell deficiency as a major part of its pathophysiological mechanism. Hence, protection of pancreatic beta-cells is a potential therapeutic strategy [5]. Vildagliptin, as a DPP-4 inhibitor, is one of the antidiabetic drugs that can overcome the existing problem of the increasingly prevalent diabetic disease [2].

This study was an attempt to evaluate the effect of vildagliptin on some biochemical and histopathological parameters in male Wistar rats and explore the possible role of vildagliptin against the destruction of the pancreatic beta-cell in type 2 diabetic rats.

In this study, we used streptozotocin-nicotinamide to induce a type 2 diabetic rat model, in which administration of nicotinamide affords partial protection of beta-cells against the severe cytotoxicity of STZ, which is an antibiotic that causes pancreatic islet beta-cells destruction. This regimen produced an insulin-deficient type 2 diabetes model characterized by stable, moderate hyperglycemia and associated with a 60% loss of beta-cell function [24].

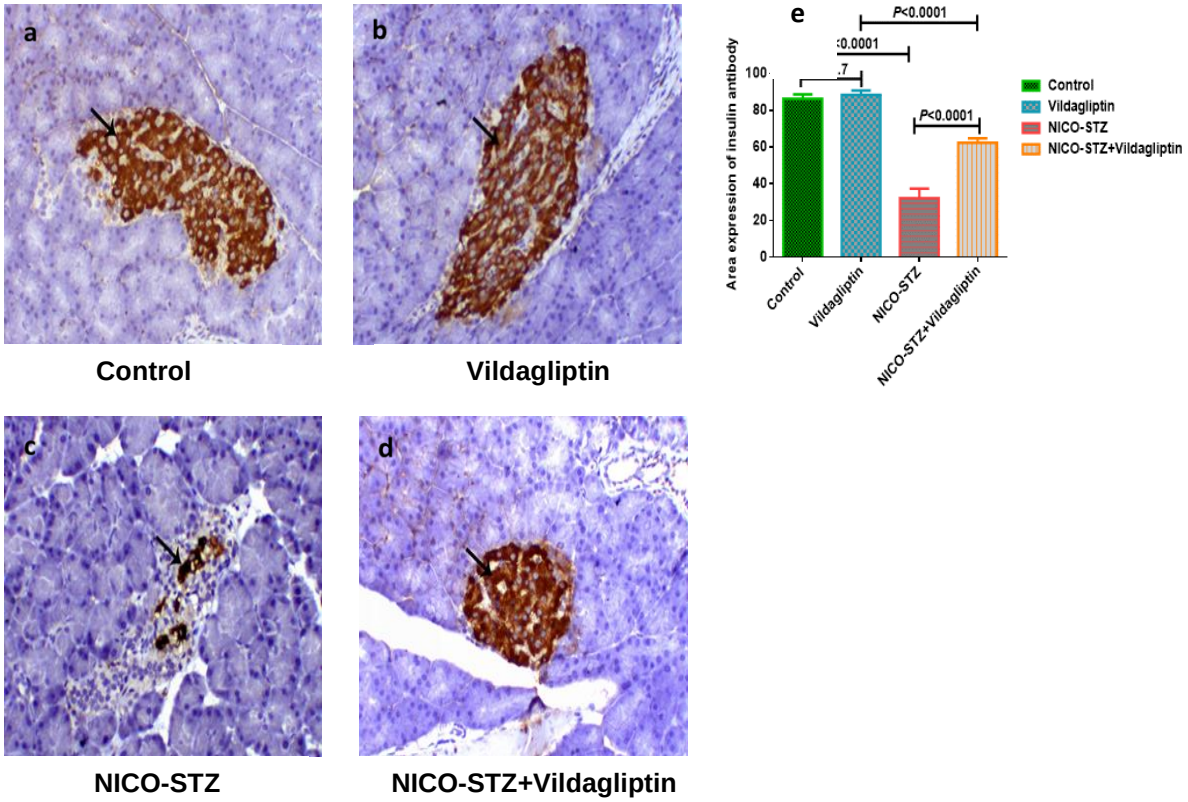

Figure 6. Immunohistochemical staining (IHC) of pancreatic sections with anti-insulin antibody declared the effect of vildagliptin on proliferation in islets of (a) normal control rats, (b) normal rats treated with vildagliptin, (c) diabetic rats, (d) diabetic rats treated with vildagliptin, (e) Quantification percentage of positive area of expression of insulin antibody in pancreatic beta-cell of normal and diabetic rats.

In our study, we demonstrated that the treatment with vildagliptin improved pancreatic beta-cell function in diabetic rats [25]. This is clearly shown by the modest reduction of blood glucose and significant increase of plasma insulin level after treatment of the diabetic rats with vildagliptin, which reflected an improvement of pancreatic beta-cell function[26], which indirectly contributes to the preservation of pancreatic beta-cells as hyperglycemia is known to make a major contribution in pancreatic beta-cells apoptosis. Our obtained results agreed with the findings reported in the previous study [27].

Furthermore, our study showed that treatment with vildagliptin reduced glucagon levels, which might improve glycemic control. These results agreed with a clinical study demonstrating that vildagliptin treatment could inhibit glucagon secretion [28].

Our study also showed that the treatment with vildagliptin increased plasma active GLP-1 concentrations, contributing to the beta-cell effects in the current study. These obtained results agreed with the studies that demonstrated that vildagliptin prolongs the glucagon-like peptide's half-life, leading to subsequent suppression of glucagon secretion[29] and stimulation of insulin [26].

Data presented in our study demonstrated that treatment with vildagliptin for 4 weeks preserved beta-cells in many ways. Firstly, our study showed that there is an effect of

vildagliptin to preserve pancreatic beta-cells in diabetic rats through its ability to reduce oxidative stress, as it is known from previous studies that prolonged untreated oxidative stress in diabetes leads to endothelial beta-cell dysfunction, reduction of insulin production, impaired insulin secretion, and its apoptosis [28]. Data showed a reduction in the levels of MDA and a significant elevation in the activities of SOD and CAT in diabetic rates after treatment with vildagliptin relative to untreated rats. Our obtained results agreed with the findings that vildagliptin has a significant antioxidant effect by reversing oxidative stress and endoplasmic reticulum stress [27,30]. As a result, the treatment with vildagliptin enhances pancreatic beta-cell function and insulin sensitivity and reduces lipid peroxidation [31].

The other way to protect pancreatic beta-cells is to avoid beta-cell loss, including inhibiting beta-cell apoptosis, necrosis, and dedifferentiation [5]. Results obtained in our study indicated that the treatment with vildagliptin for 4 weeks significantly increased viability percentages and decreased both necrosis and late apoptosis with non-significantly decreased percentages of early apoptosis in diabetic rats compared to untreated diabetic rats.

Also, vildagliptin enhanced beta-cell differentiation and proliferation [32] and decreased apoptosis [33]. This agreed with the results obtained from both histopathological examinations of the pancreatic islet, which showed that vildagliptin decreased the apoptosis to mild or moderate degenerative lesions associated with macrophages infiltration in diabetic rats. The results obtained from immunohistochemical examination of the pancreatic islet showed that treatment with vildagliptin resulted in a marked increase of insulin expression within the beta-islets of Langerhans and anti-insulin antibodies in diabetic rats. All this confirmed the preservation of pancreatic beta-cell mass in diabetic rats after treatment with vildagliptin for 4 weeks.

4. Conclusions

In conclusion, we demonstrated that vildagliptin as a Dpp4 inhibitor enhanced pancreatic beta-cell function and mass by alleviating pancreatic beta-cell apoptosis and oxidative stress or improving overall glycemic control. Further study is needed to explore whether other mechanisms are also involved in vildagliptin's protective effect on pancreatic beta-cells.

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Conflicts of Interest

The authors declare no conflict of interest.

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