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Pancreatic β- cell Effects of Canagliflozin versus Vildagliptin on Streptozotocin-Induced Type 2 Diabetes Mellitus in Male Albino Rats

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Abstract

Background: Diabetes mellitus (DM) is a chronic illness characterized by an elevated blood glucose associated with absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action.

Aim: This study aimed to compare the pancreatic β-cell function improvement by DPP-4 inhibitors as Vildagliptin and SGLT2 inhibitors as Canagliflozin on diabetic male albino rats.

Methods: Thirty-six rats were randomly divided into 4 equal groups (n=9): control group, untreated diabetic group, vildagliptin-treated diabetic group (20 mg/kg/day by orally) and canagliflozin-treated diabetic group (40 mg/kg/day orally). Type 2 DM was induced by intraperitoneal(IP) injection of nicotinamide (230 mg/kg) 15 min prior to single dose injection of streptozotocin (60 mg/kg, I.P.). The assessed parameters were fasting blood glucose (FBG), proinsulin, insulin, proinsulin/Insulin ratio, relative expression of *MafA* gene (β-cells specific transcription factor), *Pancreatic and duodenal homebox-1 (PDX-1)* gene and histopathology for pancreatic β-cell.

Results: The results of the present study demonstrated that oral administration of vildagliptin orally for 4 weeks for untreated diabetic group improved pancreatic β-cell function as evidenced by the significant reduction of the proinsulin/insulin ratio, the significant increase of *MafA* and *PDX-1* gene expression and the improvement of the histopathological picture of the pancreatic β-cell.

On the other hand, the results of the present study showed that oral administration of canagliflozin orally for 4 weeks for untreated diabetic group significantly increased insulin and proinsulin with no significant change of proinsulin/insulin ratio. Also, canagliflozin improved pancreatic β-cell function as evidenced by the significant increase of *MafA* and *PDX-1* gene expression and the improvement of the histopathological picture of the pancreatic β-cell. However, the effects of vildagliptin on these pancreatic β-cell parameters were significantly higher than that of Canagliflozin .

Conclusion: Oral vildagliptin produces greater improvement of the β-cell secretory function than canagliflozin and thus it can be favourably recommended for type 2 diabetic patients .

Keywords: Diabetes mellitus, Vildagliptin, Canagliflozin, Insulin.

I. INTRODUCTION

Diabetes mellitus (DM) is a chronic illness describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs **[1].**

Insulin is the key hormone for regulation of blood glucose. Normoglycemia is maintained by the balance between insulin action and insulin secretion. The normal pancreatic β-cell can adapt to changes in insulin action; a decrease in insulin action is accompanied by upregulation of insulin secretion (and vice versa). Thus, β-cell dysfunction is a critical component in the pathogenesis of Type 2 DM (T2DM) **[2].**

Vildagliptin is an orally active, potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor, shown to be effective and well-tolerated in patients with T2DM as either monotherapy or in combination with other anti-diabetic agents **[3].**

Canagliflozin is a highly selective SGLT2 inhibitor that inhibits reabsorption of filtered glucose in the proximal tubules of the kidney and lowers the renal threshold for glucose, thereby increasing urinary glucose excretion **[4].** The increased urinary glucose excretion also produces a loss of calories associated with a reduction in bodyweight, as well as an osmotic diuretic effect, which leads to modest reductions in blood pressure **[5].**

II. MATERIAL AND METHODS

***Animals**

The study was done on a total number of 36 adult male albino rats weighing 200-250 gm. Rats purchased from the animal house of Faculty of Veterinary Medicine, Zagazig University, Egypt. All experiments of this study were done according to the guidelines of animal research. The animals were on a raised mesh bottoms cages for preventing coprophagy. Standard food was allowed ad libitum and tap water was freely accessed at room temperature ranging between 20-26˚C with 12 hours light/dark cycle. Animals were left for one week prior to the beginning of the study to accommodate the environment.

***Drugs and Chemicals**

Streptozotocin [STZ] (Sigma–Aldrich, St. Louis, MO), Nicotinamide (E.I.P.I.Co.A.R.E), Canagliflozin (Janssen Pharmaceuticals Inc., UK) and Vildagliptin (Novartis, Novartis Pharma AG,Basel, Switzerland). All drugs were supplied in powder form and were freshly prepared in normal saline solution before administration.

Induction of type 2 diabetes mellitus

Type 2 DM was induced in 27 rats by fasting them overnight, then intraperitoneal (I.P.) injection of nicotinamide (230 mg/kg) 15 minutes prior to single dose injection of streptozotocin (60 mg/kg, I.P.) freshly dissolved in sodium citrate buffer (pH 4.5). Control rats received equal volumes of saline I.P. Then, rats were given glucose 5% in the drinking water for 24 hours to avoid hypoglycemia **[6].**

After two weeks of induction of T2DM, fasting blood glucose level was measured for rats and those with moderate hyperglycemia (145-221 mg/dl) were considered diabetic and included in the study, they were maintained on ordinary chow diet throughout the experiment **[7].**

***Experimental design**

After induction of T2DM, rats were randomly divided into four groups (9 rats/group) : **group 1(C) :** control group , " non-diabetic rats" ; **group 2(D) :** untreated diabetic rats , **group 3(D+VILD) :** vildagliptin-treated diabetic rats that received 20 mg/kg/day orally for 4 weeks **[8]** and **group 4 (D+CAN) :** Canagliflozin-treated diabetic rats received (40 mg/kg/day orally for 4 weeks) **[9].** Group 1 and 2 received 0.5 ml saline daily orally for 4 weeks. At the end of experiments , blood samples were collected for biochemical assays and pancreatic samples were collected for biochemical assays and histopathological examination.

Fasting blood glucose level determination :

Fasting blood glucose (FBG) level was measured after induction of T2DM , on the last day of the experiments, using Blood Glucose Meter (Accu-Chek; Roche Diagnostics, Mannheim, Germany). One drop of blood was obtained by tail vein puncture **[10].**

Blood and pancreatic tissue sampling:

Blood samples were obtained for biochemical studies by means of capillary glass tubing from retro-orbital plexus of rats under diethyl ether anesthesia by the procedures described by **Sloboda et al. [11].** Subsequently, each rat was sacrificed and the pancreatic tissue was excised, cleared of fat and lymph nodes, washed with ice-cold saline and divided into two parts: one part was fixed in 10% buffered formalin and embedded in paraffin for histopathological studies, while the other part was washed with ice-cold saline, immersed immediately in liquid nitrogen and kept at −80 °C for biochemical studies **[12].**

Serum insulin and proinsulin level determination:

Serum insulin and proinsulin levels were measured using ELISA Kits for insulin and proinsulin assays supplied by RayBiotech, USA **[13].**

Quantitative real time reverse transcription-polymerase chain reaction (RT-PCR) analysis of pancreatic tissues to measure *MafA* gene (β-cell transcription factor) and *pancreatic and duodenal homobox-1 (PDX-1)* gene **[14].**

Primer sequence (5′-3′) for *MafA* and *PDX-1* genes:

MafA gene **Forward:** TTCAGCAAGGAGGAGGTCAT
Reverse: CCGCCAACTTCTCGTATTTC

PDX-1 gene **Forward:** CATCTCCCCATACGAAGTGC
Reverse: GGGGCCGGGAGATGTATTTG

Histopathological study for pancreas:

The pancreatic tissues of rats were fixed in buffered 10% formalin solution and then embedded in a paraffin wax. Tissues were then sectioned at 5-µm, stained with hematoxylin-eosin (H&E) in standard histological manner and observed under light microscope to assess morphological changes **[12].**

Statistical analysis

One-way analysis of variance (ANOVA) was used for comparison of all groups. Least significant difference (LSD) was used for comparison of groups. All data were expressed as mean ± standard error (SE). Significance was accepted at p-values < 0.05. The collected data were analyzed by computer using Statistical Package of Social Services version 25 (SPSS) **[15].**

III. RESULTS:

Effects on fasting blood glucose level :

After induction of T2DM, FBG levels of untreated and treated diabetic groups were significantly higher than the control group. At the end of experiments, untreated diabetic group showed significant increase of FBG as compared to normal control ,Vildagliptin-treated and Canagliflozin- treated groups . There was no significant difference of FBG between Vildagliptin-treated diabetic and Canagliflozin-treated diabetic groups; but the level for both groups were significantly lower than that of diabetic group and insignificantly different from control group (Table 1).

Table (1): Effect of drugs on FBG (mg/dl) for different groups:

	C	D	D+VILD	D+CAN
After induction of T2DM	84.50±3.45^{A}	209.8±9.61^{B}	205.5±8.37^{B}	215.7±13.41^{B}
At the end of experiment	84.67±3.43^{A}	238.5±7.82^{B}	85.67±3.10^{A}	89.83±3.57^{A}

Number of animals = 9 rats in each group. Values represent mean ± SE.
Within the same row, values without common superscript capital letters are significantly different (p<0.05).

Effects on serum insulin level:

Untreated Diabetic group showed significant decrease of serum insulin level as compared to control group. The treated groups showed significant increase of serum insulin level as compared to untreated diabetic group; but those values were still significantly lower than that of control group. However, value of vildagliptin-treated group was significantly higher than that of canagliflozin-treated group (Table 2).

Effects on serum proinsulin level:

Untreated and treated Diabetic groups showed significant decrease of serum proinsulin level as compared to control group. The level for vildagliptin-treated group was insignificantly different from untreated diabetic group .Meanwhile, that of canagliflozin-treated group was significantly higher (Table 2).

Effects on proinsulin/insulin ratio:

Untreated Diabetic group showed significant increase of proinsulin/insulin ratio as compared to the control group and Vildagliptin- treated group.Meanwhile , Canagliflozin-treated group showed no significant change as compared to diabetic group (Table 2).

Table (2): The Effect of drugs on serum level of insulin, proinsulin and proinsulin/insulin ratio in different groups:

	C	D	D+VILD	D+CAN
Insulin (µIU/ml)	28.68±1.73^{A}	8.03±0.46^{B}	17.19±0.67^{C}	11.95±0.61^{D}
Proinsulin (pMOL/ml)	14.02±0.74^{A}	5.10±0.37^{B}	6.25±0.35^{BC}	7.65±0.39^{C}
Proinsulin /Insulin ratio	0.49±0.03^{A}	0.63±0.02^{B}	0.38±0.01^{C}	0.65±0.02^{B}

Number of animals = 9 rats in each group. Values represent mean ± SE.
Within the same row, values without common superscript capital letters are significantly different (p<0.05).

Effects on *MafA* and *PDX-1* genes level:

Untreated Diabetic group showed significant decrease of relative expression of *MafA* and *PDX-1* genes as compared to the control group. The treated groups showed significantly higher values than untreated group , with higher significant value for Vildagliptin- treated group over Canagliflizin- treated one . (Table 3).

Table (3): Effect of drugs on relative expression of *MafA* and *PDX-1* genes in pancreatic tissue of different groups:

	C	D	D+VILD	D+CAN
MafA level	1.07 ± 0.048^{A}	0.29 ± 0.022^{B}	0.81 ± 0.030^{C}	0.49 ± 0.032^{D}
PDX-1 level	1.05 ± 0.0198^{A}	0.26 ± 0.026^{B}	0.86 ± 0.028^{C}	0.58 ± 0.024^{D}

Number of animals = 9 rats in each group. Values represent mean ± SE.
Within the same row, values without common superscript capital letters are significantly different ($p<0.05$).

Histopathological results:

Pancreatic section for the ***control group*** showed normal islets of Langerhans with granulated cytoplasm and regular lining of acinar cells around the islets (Figure 1), that for ***Untreated diabetic group*** showed marked reduction of size of islets of Langerhans with loss of most β-cells, cytoplasmic degeneration, areas of hemorrhage with irregular lining of acinar cells around the islets (Figure 2). ***Vildagliptin-treated group*** pancreatic section showed increase of mass of islets of Langerhans with regular lining of acinar cells around the islets (Figure 3). Meanwhile, ***Canagliflozin-treated group*** showed marked increase of mass of islets of Langerhans (increased number and size of islets cells) with irregular lining of acinar cells around the islets, minimal hemorrhage (Figure 4).

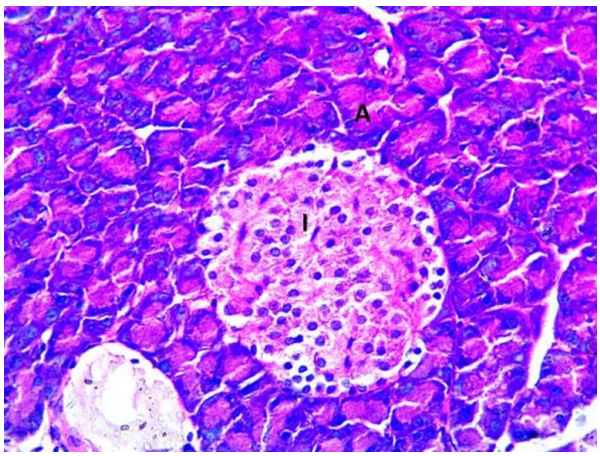

Figure (1): Control (Non-Diabetic) Group (C): Normal islets of Langerhans (I) with granulated cytoplasm and regular lining of acinar cells (A) around the islets, (H&E stain, x400)

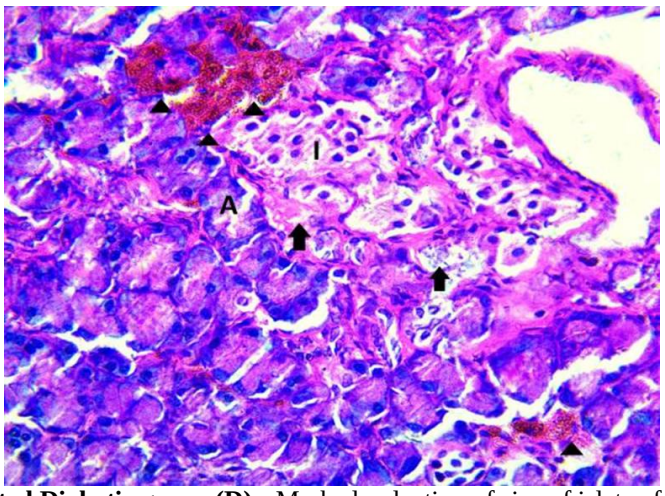

Figure (2): Untreated Diabetic group (D): Marked reduction of size of islets of Langerhans (I) with cytoplasmic degeneration (arrows), areas of hemorrhage (arrowheads), with irregular lining of acinar cells (A) around the islets. (H&E stain, x400).

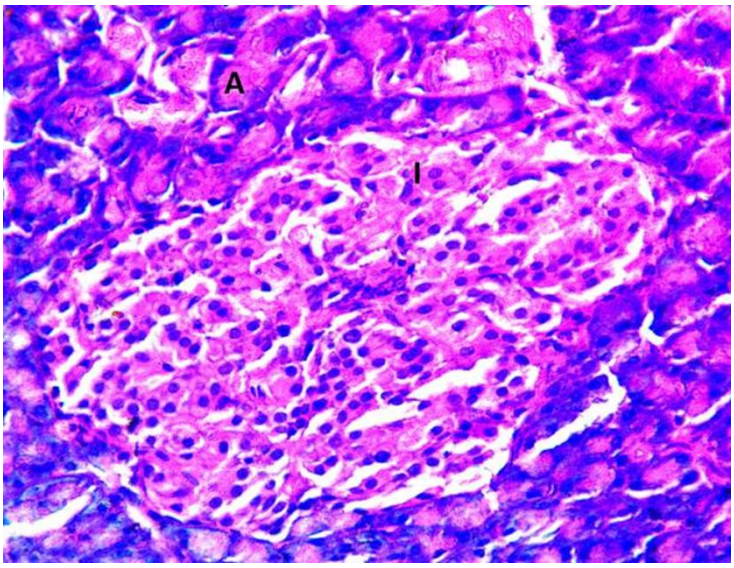

Figure (3): Vildagliptin-treated group (D+VILD): Increase of mass of islets of Langerhans (I) (increased number and size of islets cells) with regular lining of acinar cells (A) around the islets, (H&E stain, x400)

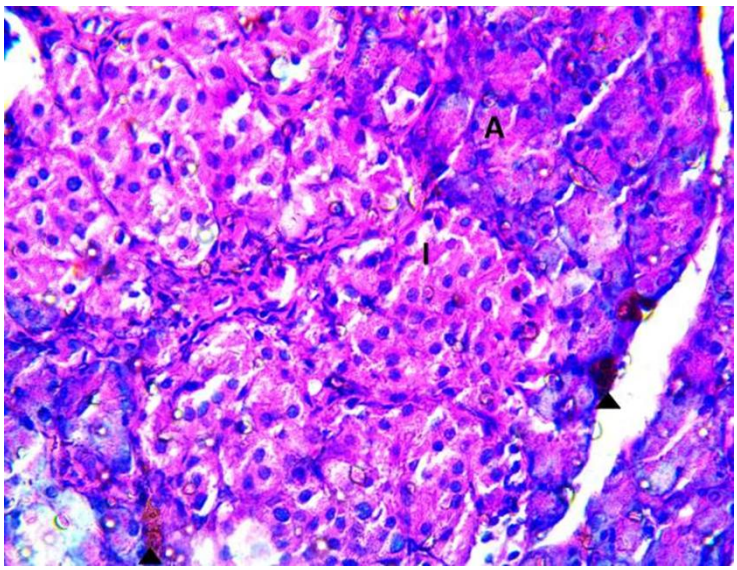

Figure (4): Canagliflozin-treated group (D+CAN): Marked increase of mass of islets of Langerhans (I) (increased number and size of islets cells) with irregular lining of acinar cells (A) around the islets, minimal hemorrhage (arrowheads) (H&E stain, x400)

IV. DISCUSSION:

In the present study, T2DM model achieved by intraperitoneal injection of nicotinamide (230 mg/kg) 15 min prior to single dose intraperitoneal injection of streptozotocin (60 mg/kg) freshly dissolved in sodium citrate buffer this developed moderate stable hyperglycemia and explained by **Szkudelski [16]** who observed that this method was described by 40% reduction in the mass of β-cell resultant in hypoinsulinemia and moderate stable hyperglycemia **[17].**

The results of the present study revealed that administration of vildagliptin lowered FBG level of diabetic rats. **Gallwitz [18]** stated that the DPP-4 inhibitors show a high efficacy concerning inhibition of DPP-4 which successively leads to elevation of post-prandial GLP-1 plasma concentrations , mediates the glucose-dependent stimulation of insulin secretion and inhibition of glucagon secretion **[19].** Moreover, the local inhibitory effect of DPP-4 inhibitors on GLP-1 degradation in the intestinal mucosa may contribute to favorable metabolic regulation by stimulating the autonomic afferent nervous system **[20].**

The current study showed that administration of canagliflozin significantly lowered FBG of diabetic rats. **Ali et al. [21]** reported that rats given canagliflozin (10 or 25 mg/kg, by oral gavage) for 35 consecutive days recorded a decreased level of FBG . This is assumed to be explained by the fact that Canagliflozin act on

kidneys to lower the renal threshold for glucose and enhance the urinary glucose excretion and reduce hyperglycemia **[22, 23].**

The results of the current study demonstrated that untreated diabetic group produces significant decrease of serum insulin and proinsulin levels with significant increase of serum proinsulin/insulin ratio. **Ohkura et al. [24]** stated that the significant increase of serum proinsulin/insulin ratio for patients with T2DM reveals reduction of insulin secretory capability of pancreatic β-cells due to β-cell damage.

The results of the current work demonstrated that administration of vildagliptin showed significant increase of insulin level with no significant change for proinsulin level and consequently significant decrease of proinsulin/insulin ratio of diabetic hypertensive rats. These findings are in accordance with **Ferrannini et al. [25]** who reported an association between fasting glucagon and proinsulin levels for patients with insulin resistance. An association was observed between postprandial glucagon and proinsulin concentrations in patients treated with DPP-4 inhibitors. Several studies have suggested an association between increased intact proinsulin levels and the development of cardiovascular complications for diabetic and non-diabetic subjects **[26].**

In the present study comparing the effects of canagliflozin versus vildagliptin on proinsulin/insulin ratio revealed superiority of vildagliptin over canagliflozin. **Takahashi et al. [27]** clarified this by assuming that the effects of SGLT2 inhibitors on blood glucose levels are mainly mediated by the insulin-dependent intracellular transport and by the SGLT2 inhibitor-induced amplification of urinary glucose loss. Proinsulin/insulin ratio is a valuable biomarker for comparing the effects of DPP-4 inhibitors with SGLT2 inhibitors on pancreatic β- cell function. **Tsurutani et al. [28]** reported that after treatment with 50 mg of either Ipragliflozin (SGLT2 inhibitor) or Sitagliptin (DPP-4 inhibitor) once daily, proinsulin/insulin ratio was decreased for the Sitagliptin group as compared with Ipragliflozin group and this showed that the effect of Sitagliptin on β-cell function may help to recognize the ideal antidiabetic agent for each patient. For instance, Sitagliptin might be more beneficial for those with insulin secretory dysfunction and Ipragliflozin for those with excessive fat and insulin resistance **[29].**

The results of the current study revealed that untreated diabetic group produces significant decrease of *MafA* gene and *PDX-1* levels. **Okauchi et al. [30]** related this to the decrease of insulin content as it is known that insulin gene transcription factors such as *MafA* and *PDX-1* are very important to preserve mature β-cell function and insulin gene transcription.

The results of the present study showed that administration of vildagliptin produced increase of *MafA* gene and *PDX-1* levels. **Shinjo et al. [31]** stated that Anagliptin (DPP4 inhibitor) administration resulted in up-regulation of mRNA levels of the β-cell markers *PDX-1* and *MafA* demonstrating that Anagliptin reversed the deterioration of islets of Langerhans of diabetic mice. The increased active GLP-1 functions of β-cells, promoting their propagation in response to DPP-4 inhibitor administration resulting in improved β-cell functions such as insulin secretion **[32].**

Moreover, the effects of Canagliflozin on *MafA* and *PDX-1* are in agreement with **Okauchi et al. [30]** who reported that mice given luseogliflozin (SGLT2 inhibitor) 0.0025% and luseogliflozin 0.01% in chow showed significant increase **of** the *MafA* and *PDX-1* levels. The increased expression of insulin mRNA levels explains the increased insulin biosynthesis and secretion.

The histopathological results of the present study presented that diabetic group showed reduction **of** size of islets of Langerhans. **Eizirik et al. [33]** identified that the histology of pancreatic islets **of** T2DM is due to loss of functional β-cell mass in patients with T2DM.

Moreover, the histopathological results of vildagliptin were in line with **Dobrian et al.** and **Takeda et al. [34, 35]** who related these findings to increased β-cell proliferation, decreased β-cell apoptosis and suppression of islet inflammation by DPP-4 inhibitors.

In addition, the histopathological findings of canagliflozin were in agreement with those obtained by **Kaneto et al. [36]** who stated that pancreatic β-cell mass was larger which may be due to increased β-cell proliferation and decreased apoptosis.

V. CONCLUSION

Oral vildagliptin produces greater improvement of the β-cell secretory function than canagliflozin and thus it can be favourably recommended to use vildaglipti for type 2 diabetic patients .

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