

Comparative Study on the Cardiovascular and Pancreatic Effects of Canagliflozin versus Vildagliptin on Experimentally Induced Diabetes and Hypertension in Male Albino Rats

Tarteel Youssef Hassan Mohamed ¹, Mohamed Abdelhafiez Ahmed ¹, Shireen S. Mahmoud ¹

¹Department of Clinical Pharmacology, Faculty of Medicine, Zagazig University, Egypt,

Corresponding author: Tarteel Youssef Hassan Mohamed.

E-mail: tarteelyoussef2116@gmail.com

Abstract

Background: Diabetes mellitus and hypertension are interrelated diseases sharing common etiology and disease mechanisms that predispose to cardiovascular disease. Hypertension is approximately twice frequent in diabetic patients compared by non-diabetics.

Aim: This study was designed to compare the potential cardiovascular beneficial effects and pancreatic β -cell function improvement by vildagliptin as DPP-4 inhibitor and canagliflozin as SGLT2 inhibitor on diabetic hypertensive male albino rats.

Materials and Methods: thirty-six rats were randomly divided into 4 equal groups: Control group, Diabetic hypertensive, Vildagliptin-treated diabetic hypertensive (20 mg/kg/day by orally) and Canagliflozin-treated diabetic hypertensive groups (40 mg/kg/day orally). T2DM was induced by I.P. injection of nicotinamide (230 mg/kg) 15 min prior to single dose injection of streptozotocin (60 mg/kg, IP). Hypertension was induced by L-NAME (50 mg/kg, PO) for 4 weeks. The assessed parameters were systolic and diastolic blood pressure (SBP and DBP), fasting blood glucose (FBG), serum endothelial nitric oxide synthase (eNOS), proinsulin, insulin, proinsulin/Insulin ratio, relative expression of MafA gene (β -cells specific transcription factor), Pancreatic and duodenal homeobox-1 (PDX-1) gene, and histopathology for pancreas.

Results: The results of the present study demonstrated that oral administration of vildagliptin orally for 4 weeks for diabetic hypertensive rats produced beneficial cardiovascular effects as evidenced by the significant reduction of SBP and DBP and the significant increase in serum eNOS level. Moreover, vildagliptin improved pancreatic β -cell function 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 pancreas. On the other hand, the results of the present study showed that oral administration of canagliflozin orally for 4 weeks for diabetic hypertensive rats produced significant reduction of SBP, but there is insignificant change of DBP and serum eNOS level. Also, it was found that oral administration of canagliflozin significantly increased insulin and proinsulin with no significant change of proinsulin/insulin ratio. In addition, canagliflozin improved pancreatic β -cell function evidenced by the significant increase of MafA and PDX-1 gene expression and the improvement of the histopathological picture of the pancreas; however, vildagliptin has better effects on these pancreatic β -cell parameters.

Conclusion: Oral vildagliptin has beneficial cardiovascular effects against hypertension and improves the β -cell secretory function. These effects are greater than those of canagliflozin and

thus it can be recommended to use vildagliptin rather than canagliflozin in diabetic patients with co-existing hypertension.

Introduction

Diabetes mellitus (DM) and hypertension (HPN) are interrelated diseases that predispose to cardiovascular disease. HPN is approximately twice frequent in diabetic patients compared by non-diabetics. Lifestyle and genetic factors play an important role on both HPN and DM. Several anatomic and functional abnormalities of the vascular endothelium are associated with both DM and HPN (1).

Hyperglycemia alters endothelial cell matrix production, which may contribute to basement membrane thickening. Furthermore, hyperglycemia rises endothelial cell collagen and fibronectin synthesis with increases the activity of enzymes intricate in collagen synthesis. Hyperglycemia also delays cell replication and increases endothelial cell death in part by enhancing oxidation and glycation (2).

Vildagliptin is an oral antidiabetic drugs which act by selective inhibition of dipeptidyl peptidase-4 (DPP-4) enzyme that is responsible for degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). They improve the function of pancreatic islets of Langerhans in patients with type 2 diabetes mellitus (T2DM) through increasing both α - and β -cell responsiveness to glucose (3). DPP-4 is also found on endothelial cells of the cardiovascular system and thus, DPP-4 inhibitors improve endothelium-dependent vasodilatation, so they decrease blood pressure (4).

Canagliflozin is a recent SGLT2 inhibitor (5). It blocks renal proximal tubular reabsorption of glucose, so increases urinary glucose and sodium excretion (6). Moreover, SGLT2 inhibitors produce β -cell protective effects through increasing β -cell mass by reduction of β -cell apoptosis and enhancement of β -cell proliferation (7).

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 in 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), N(ω)-nitro-L-arginine methyl ester [L-NAME] (Sigma–Aldrich, St. Louis, MO, USA), 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

It was induced in 27 rats by fasting them overnight, then I.P. injection of nicotinamide (230 mg/kg) 15 min prior to single dose injection of streptozotocin (60 mg/kg, IP) 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 (8).

After two weeks of injection, the fasting blood glucose level was measured for rats in this group. Rats with moderate hyperglycemia (145-221 mg/dl) are considered diabetic and included in the study and were maintained on ordinary chow diet throughout the experiment (9).

Induction of hypertension

Hypertension was induced to diabetic rats through administration of L-NAME (50 mg/kg, orally) dissolved in distilled water daily for 4 successive weeks (10). Control rats received 0.5 ml saline orally for 4 weeks.

Experimental design

After induction of T2DM and HPN, rats were randomly divided into four groups (9 rats/group): **group 1:** control group (C) non-diabetic non-hypertensive rats received 0.5 ml saline solution orally daily for 4 weeks; **group 2:** (DH) diabetic hypertensive rats received 0.5 ml saline solution orally daily for 4 weeks; **group 3:** Vildagliptin-treated diabetic hypertensive rats (DH+VILD) received 20mg/kg/day orally for 4 weeks (11) and **group 4:** Canagliflozin-treated diabetic hypertensive rats (DH+CAN) received 40mg/kg/day orally for 4 weeks (12). At the end of experiment, blood samples were collected for biochemical assays and pancreatic samples were collected for biochemical assays and histopathological examination.

Determination of fasting blood glucose level:

Fasting blood glucose (FBG) level was measured after induction of T2DM and on the last day of the experiment using Blood Glucose Meter (Accu-Chek; Roche Diagnostics, Mannheim, Germany). One drop of blood was obtained by tail vein puncture (13).

Determination of systolic and diastolic blood pressure

Systolic and diastolic blood pressure (SBP and DBP) were measured after induction of HPN by L-NAME and at the last day of the experiment using 8-Channel Non-Invasive Blood Pressure Monitor (NIBP-8): Columbus, Ohio 43204, USA (14).

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 Slododa et al. (15). Subsequently, each rat was sacrificed and the pancreatic tissue was excised, cleared of fat and lymph nodes, washed with ice-cold saline and cut into two equal 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 (16).

Determination of serum endothelial Nitric oxide synthase (eNOS/NOS3)

Endothelial Nitric oxide synthase was measured using ELISA Kits for endothelial Nitric oxide synthase (eNOS/NOS3) assay supplied by Cusabio, Houston, USA (17).

Determination of serum insulin and proinsulin levels

Serum insulin and proinsulin levels were measured using ELISA Kits for insulin and proinsulin assays supplied by RayBiotech, USA (18).

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 (19). All Primers are listed in Table 1.

Table (1): Primer sequence for MafA gene and PDX-1 gene:

	Primer sequence (5'-3')
MafA gene	Forward: TTCAGCAAGGAGGAGGTCAT
	Reverse: CCGCCAACCTCTCGTATTTTC
PDX-1 gene	Forward: CATCTCCCCATACGAAGTGC
	Reverse: GGGGCCGGGAGATGTATTTG

Histopathological study for pancreas:

The pancreas 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 (16).

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 are expressed as mean \pm 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) (20).

Results

Effects on fasting blood glucose

After induction of T2DM, FBG levels in untreated and treated diabetic hypertensive rats were significantly higher than the control group. At the end of experiment, diabetic hypertensive group showed significant increase in FBG as compared to normal control group. There was no significant difference in FBG between vildagliptin-treated diabetic hypertensive and canagliflozin-treated diabetic hypertensive groups, but FBG levels in both groups were significantly lower than that of diabetic hypertensive group (Table 2).

Table (2): Effect of drugs on FBG (mg/dl) in different groups of rats:

	C	DH	DH+VILD	DH+CAN
<i>After induction of T2DM</i>	84.50±3.45 ^A	203.3±8.41 ^B	206.2±10.96 ^B	206.8±11.38 ^B
<i>At the end of experiment</i>	84.67±3.43 ^A	241.5±6.99 ^B	85.33±3.45 ^A	91.0±2.82 ^A

Number of animals = 9 rats in each group; Values represent mean ± standard error

Within the same row, values without common superscript capital letters are significantly different (p<0.05).

Effects on systolic and diastolic blood pressure

After induction of HPN by L-NAME, SBP and DBP values in untreated and treated diabetic hypertensive rats were significantly higher than the control group.

At the end of the experiment, diabetic hypertensive group showed significant increase of SBP and DBP as compared to the control group. There was no significant difference in SBP values between vildagliptin and canagliflozin-treated diabetic hypertensive groups, but both were significantly lower than that of the diabetic hypertensive group. Moreover, the DBP value of vildagliptin-treated diabetic hypertensive group was significantly lower than that of diabetic hypertensive group (Table 3).

Table (3): Effect of drugs on SBP and DBP (mmHg) in different groups of rats:

		C	DH	DH+VILD	DH+CAN
SBP	<i>After induction of HPN</i>	127.5±2.72 ^A	164.1±3.34 ^B	161.5±3.28 ^B	159.5±3.10 ^B
	<i>At the end of experiment</i>	127.9±2.98 ^A	172.8±3.77 ^B	150.1±3.28 ^C	147.9±3.68 ^C
DBP	<i>After induction of HPN</i>	78.0±2.42 ^A	99.15±2.91 ^B	103.2±3.16 ^B	101.3±3.09 ^B
	<i>At the end of experiment</i>	75.77±3.35 ^A	103.6±1.79 ^B	92.22±2.22 ^C	99.13±2.38 ^{BC}

Number of animals = 9 rats in each group; Values represent mean ± standard error

Within the same row, values without common superscript capital letters are significantly different (p<0.05).

Effect on serum endothelial Nitric oxide synthase (eNOS/NOS3)

Diabetic hypertensive group showed significant decrease of serum eNOS level as compared to the control group. Vildagliptin treated group showed significant increase of serum eNOS level as compared to the diabetic hypertensive group. However, canagliflozin-treated group showed no significant change of serum eNOS level (Table 4).

Effect on serum insulin level

Diabetic hypertensive group showed significant decrease of serum insulin level as compared to the control group. The treated groups showed significant increase as compared to untreated diabetic hypertensive group, however, value of vildagliptin-treated group was significantly higher (Table 4).

Effect on serum proinsulin level

Diabetic hypertensive group showed significant decrease of serum proinsulin level as compared to the control group. Vildagliptin treated group showed non-significant change from diabetic hypertensive group, however canagliflozin treated group showed significant increase as compared to diabetic hypertensive group (Table 4).

Effects on proinsulin/insulin ratio

Diabetic hypertensive group showed significant increase of proinsulin/insulin ratio as compared to the control group. The proinsulin/insulin ratio of vildagliptin treated group is significantly lower than

that of diabetic hypertensive group, however canagliflozin treated group showed no significant change as compared to diabetic hypertensive group (Table 4).

Table (4): Effect of drugs on eNOS, Insulin, Proinsulin and Proinsulin/Insulin ratio: in different groups of rats:

	C	DH	DH+VILD	DH+CAN
eNOS (μIU/ml)	121.7±3.50 ^A	51.52±2.57 ^B	99.72±2.70 ^C	55.62±3.78 ^B
Insulin (μIU/ml)	28.68±1.73 ^A	8.02±0.47 ^B	17.19±0.67 ^C	11.95±0.61 ^D
Proinsulin (pMOL/ml)	14.02±0.74 ^A	5.12±0.42 ^B	6.73±0.48 ^{BC}	7.48±0.39 ^C
Proinsulin /Insulin ratio	0.49±0.03 ^A	0.64±0.02 ^B	0.39±0.01 ^C	0.63±0.03 ^B

Number of animals = 9 rats in each group; Values represent mean ± standard error

Within the same row, values without common superscript capital letters are significantly different (p<0.05).

Effects on MafA gene and PDX-1 level

Diabetic hypertensive 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 diabetic hypertensive group, however, values of vildagliptin-treated group were significantly higher (Table 5).

Table (5): Effect of drugs on relative expression of MafA and PDX-1 genes in pancreatic tissue of different groups of rats:

	C	DH	DH+VILD	DH+CAN
MafA level	1.07±0.048 ^A	0.29±0.023 ^B	0.82±0.028 ^C	0.48±0.033 ^D
PDX-1 level	1.05±0.0198 ^A	0.24±0.027 ^B	0.87±0.027 ^C	0.57±0.028 ^D

Number of animals = 9 rats in each group; Values represent mean ± standard error

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. Untreated diabetic hypertensive group showed reduced size of islets of Langerhans with loss of most β-cells, and cytoplasmic degeneration. Vildagliptin-treated diabetic hypertensive group showed increased mass (increased number and size of cells) of islets of Langerhans, cytoplasmic degeneration, and regular lining of acinar cells around the islets, minimal hemorrhage. Canagliflozin-treated diabetic hypertensive group showed restored size of islets of Langerhans (near normal size), surrounded by inflammatory infiltrate (arrowheads), acinar cells.

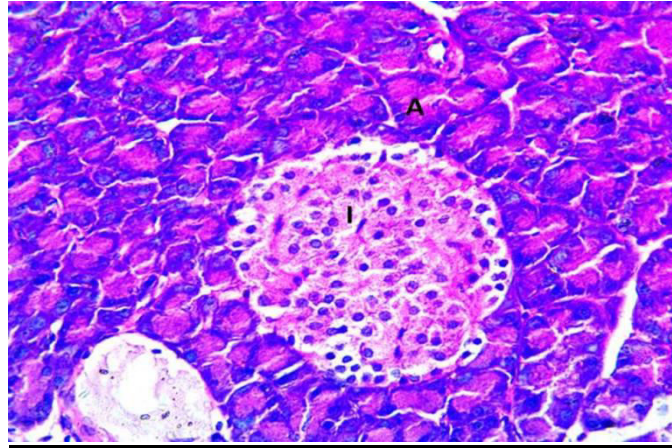


Figure (1): Control (Non-Diabetic Non-Hypertensive) Group (C): shows normal islets of Langerhans (I) with granulated cytoplasm and regular lining of acinar cells (A) around the islets, (H&E stain, x400)

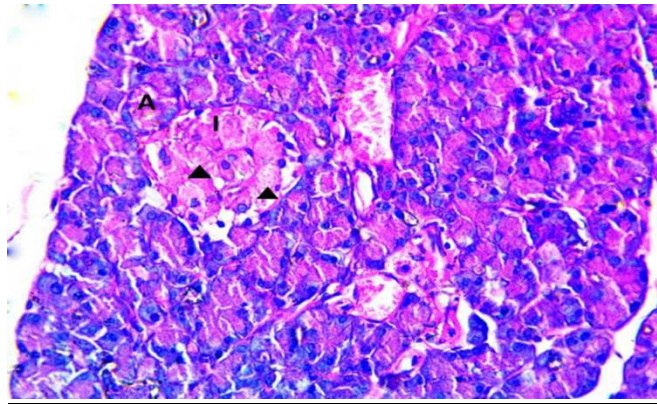


Figure (2): Diabetic hypertensive group (DH): shows reduced size of islets of Langerhans (I) with loss of most β -cells and cytoplasmic degeneration (arrowheads), acinar cells (A) (H&E stain, x400)

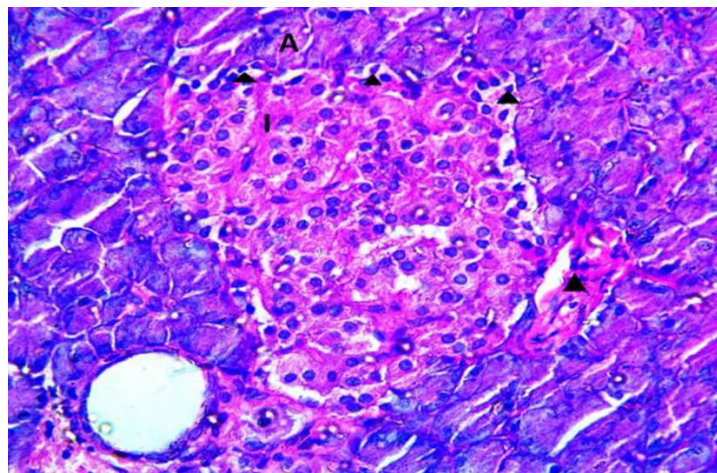


Figure (3): Vildagliptin-treated diabetic hypertensive group (DH+VILD): shows increased mass (increased number and size of islets cells) of islets of Langerhans (I), cytoplasmic degeneration (indicated by arrowheads), and acinar cells (A)

(arrows), and regular lining of acinar cells (A) around the islets, minimal hemorrhage (arrowhead) (H&E stain, x400)

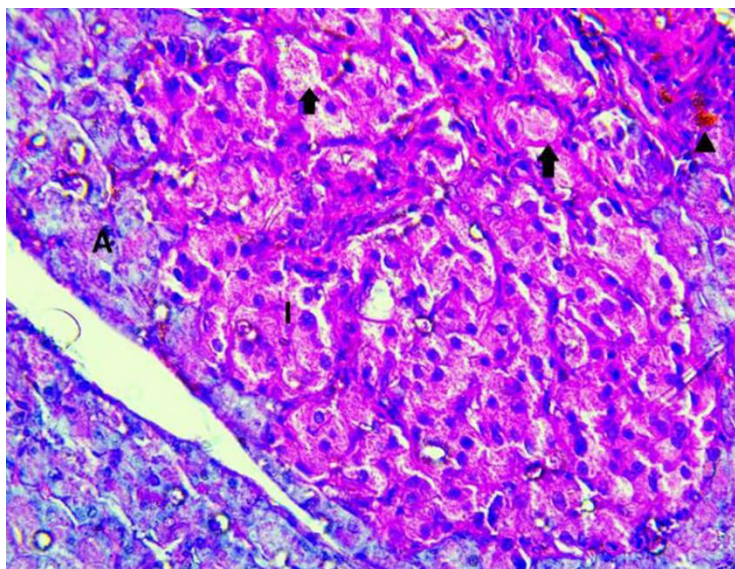


Figure (4): Canagliflozin-treated diabetic hypertensive rats (DH+CAN): shows restored size of islets of Langerhans (I) (near normal size), surrounded by inflammatory infiltrate (arrowheads), acinar cells (A) (H&E stain, x400)

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 (53)** who found that this technique was characterized by 40% reduction in β -cell mass resulting in hypoinsulinemia and moderate stable hyperglycemia (21).

The results of the present study demonstrated that vildagliptin lowered the FBG level of diabetic hypertensive rats. **Sharma et al. (22)** explained that this finding can be related to vildagliptin ability to improve insulin secretion and peripheral insulin sensitivity. Vildagliptin binds to the catalytic site of DPP-4 by covalent bond, producing prolonged enzyme inhibition. This prevents degradation of incretin hormones, specifically GLP-1 and GIP equally after ingestion of food and in the fasting state. These hormones regulate blood glucose level by enhancement of insulin release, suppression of glucagon release, decrease in appetite, and delayed gastric emptying (23). These effects reduce blood glucose level in patients with T2DM (24).

The current study showed that administration of canagliflozin significantly lowered fasting blood glucose of diabetic hypertensive rats. **Woods et al. (25)** stated that SGLT2 inhibitors renal targeting action through the ability to reduce glucose reabsorption in renal proximal tubules improving glycemic control in patients with T2DM (26).

Our results showed increase of both SBP and DBP after 4 weeks of administration of L-NAME and at the end of the experiment for untreated diabetic hypertensive rats. Similar findings were reported by **Majithiya et al. (10)** who explained that this is due to blockade to nitric oxide synthetase, increased sympathetic tone or increased renin secretion due to hyperglycemia in these rats.

Vildagliptin significantly lowered both SBP and DBP of diabetic hypertensive rats. **Bolevich et al. (27)** reported that this can be related to the fact that DPP-4 inhibition increases GLP-1, which is related to cardiac favorable lipid status (28). Also, there are DPP-4 non-incretin substrates intricate in inflammation, immunity and cardiovascular system function, their expression on endothelial surface reduces the vascular tone. Furthermore, animal studies have shown nitric oxide (NO)-dependent or independent arterial relaxation induced by GLP-1 (29). These vasodilator properties might also be mediated through GLP-1 metabolites and independently of the GLP-1 receptor, acting as an alternative to an NO/cGMP-dependent mechanism (30).

In the present work, we observed that administration of canagliflozin lowered SBP more than the DBP of diabetic hypertensive rats. **Filippatos et al. (31)** stated that empagliflozin showed drug-associated hemodynamic changes that may explain the decrease in blood pressure. **Weir et al. (32)** reported that patients with elevated SBP or DBP at baseline who received canagliflozin showed greater absolute reductions in SBP and DBP, respectively. SGLT2 inhibitors act as loop diuretics reducing intravascular volume this would be more likely to result in reductions in SBP compared with DBP. Changes in sodium excretion may also impact blood pressure lowering with canagliflozin. Moreover, experimental data published by **Tamura et al. (33)** have clearly shown that empagliflozin also decreased salt loading-mediated blood pressure elevation. SGLT2 inhibitors decreased obesity and hyperglycemia-related oxidative stress and inflammation of the vascular wall, as well as improved endothelial function through protection of endothelium from sodium overload can lead to an improvement in arterial stiffness and vascular resistance. Weight loss and visceral fat reduction related to increased diuresis and glucosuria-associated loss of calories may play a role in blood pressure lowering.

The results of our study revealed that untreated diabetic hypertensive group showed significant decrease of serum eNOS level. **Wu et al. (34)** revealed that L-NAME is a non-selective nitric oxide synthase (NOS) inhibitor and is widely used experimentally to inhibit NOS activity both in vivo and in vitro.

Our results demonstrated that vildagliptin significantly increased serum eNOS level of diabetic hypertensive rats. **Liu et al. (35)** reported that inhibition of DPP-4 enhances eNOS activity and in turn increases NO release, which produces a vasodilatory effect. Also, it improved NO bioavailability; this increase was associated with a concomitant decrease in nitroxidative stress. Also, reduction in blood pressure is attributed to improved endothelial function linked to enhanced glycemic control in hypertensive animals (36).

On the other hand, we demonstrated that canagliflozin insignificantly increased serum eNOS level, this can be related to the suggestion that hypotensive effects of canagliflozin were explained by mechanisms other than eNOS affection (31, 32).

The results of the current study demonstrated that untreated group produce significant decrease in serum insulin and proinsulin levels with significant increase in serum proinsulin/insulin ratio. **Ohkura et al. (37)** found that for patients with T2DM, there is a significant increase in serum proinsulin/insulin ratio may be due to β -cells destruction. This reflects impairment of insulin secretory capacity of β -cells in patients with T2DM.

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 showed significant decrease of proinsulin/insulin ratio of diabetic hypertensive rats. These findings are in

accordance with **Riche et al. (38)** who demonstrated that administration of sitagliptin as monotherapy or in combination produced a decrease in proinsulin/insulin ratio. This indicates improvement in the secretory and resistance profile of the β -cell (**39**). This is explained by the underlying mechanism behind incretin hormones, GLP-1 signaling directly modifies the susceptibility to apoptotic injury and provides a new potential mechanism for preservation or enhancement of β -cell mass (**40**). **Forst et al. (41)** found that this decrease in proinsulin/insulin ratio was attributed to that linagliptin mediates its effects on postprandial glucose control primarily through the inhibition of the glucagon release from the α -cell, and thereby relieves β -cell stress. DPP-4 inhibitors improve glucose-dependent insulin secretion from β -cell through the conversion of intact proinsulin into insulin and C-peptide (**42**).

In the present study comparing the effects of canagliflozin versus vildagliptin on proinsulin/insulin ratio revealed superiority of vildagliptin over canagliflozin. **Takahashi et al. (43)** explained this by that blood glucose levels are controlled not only by the insulin-dependent intracellular transport but also by the SGLT2 inhibitor-induced augmentation of urinary loss of glucose. proinsulin/insulin ratio may be a useful biomarker of pancreatic β -cell function for comparing DPP-4 inhibitors with SGLT2 inhibitors. Similar results were obtained by **Tsurutani et al. (44)** who reported that after 12 weeks of treatment with 50 mg of either ipragliflozin or sitagliptin once daily, proinsulin/insulin ratio was decreased for the sitagliptin group as compared with the ipragliflozin group this indicates the favorable effect of sitagliptin on β -cell function and may help clinicians to identify the optimal 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 (**45**).

The results of the current study revealed that untreated diabetic group produce significant decrease of MafA gene and PDX-1 levels. **Okauchi et al. (46)** explained the decrease of insulin content. It is known that insulin gene transcription factors such as MafA and PDX-1 are very important to maintain mature β -cell function and insulin gene transcription.

The results of the present study showed that administration of vildagliptin increase in MafA gene and PDX-1 levels. These results were in accordance with **Shinjo et al. (47)** who reported that anagliptin administration resulted in up-regulation of mRNA levels of the β -cell markers PDX-1 and MafA indicating that anagliptin reversed the degeneration of islets of Langerhans in streptozotocin-treated mice. Some reports have shown that increased active GLP-1 functions in β -cells, promoting their proliferation in response to DPP-4 inhibitor administration resulting in improved β -cell functions such as insulin secretion (**48**).

Moreover, canagliflozin significantly increases both MafA gene and PDX-1 levels. **Kaneto et al. (49)** reported that Luseogliflozin (SGLT2 inhibitor) exerted increased expression levels of various β -cell-related factors, including insulin and insulin gene transcription factors, such as MafA and PDX1. These indicate that SGLT2 inhibitors have beneficial effects on preserving pancreatic β -cell function.

The histopathological results of the present study showed that group showed reduction in size of islets of Langerhans. **Eizirik et al. (50)** stated that the histology of pancreatic islets in T2DM is due to the progressive loss of functional β -cell mass in patients with T2DM.

Moreover, the histopathological results of vildagliptin were in agreement with **Takeda et al. (51)** who related these findings to increased β -cell proliferation and decreased β -cell apoptosis. Also, suppression of islet inflammation by DPP-4 inhibitors has been reported **(52)**.

The histopathological findings of canagliflozin were in line with those obtained by **Kaneto et al. (49)** who reported that pancreatic β -cell mass was larger which may be due to increased β -cell proliferation and decreased β -cell apoptosis.

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