Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 07, 2021

Efficacy of Sitagliptin on Cardiovascular Responses, Antioxidant Activity and Some Metabolic Aspects among STZ Induced-Diabetic Rats

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Received: 6-2021, Revised: 7-2021, Accepted: 8-2021, e-Published: 8-2021

ABSTRACT

Diabetes mellitus type II is a metabolic disorder in glucose utilization due to inability of the body's cells to use insulin hormones. Consequently, the blood-glucose level is increasing leading to other complications, including a rise of blood cholesterol level and elevated blood pressure. The current study was intended to evaluate the efficacy and protective role of sitagliptin on diabetic adult male rats. To achieve this goal, STZ induce diabetic rats were administrated sitagliptin (10 mg/kg b.wt.) orally once daily for 30 successive days. The attained results showed a significant decrease in blood glucose, TG, cholesterol, LDL and MDA of diabetic rats treated with sitagliptin. In addition, an improvement in the levels of blood insulin H., HOMA-IR, HDL and CAT enzyme was observed compared to the diabetic non treated rats. Moreover, sitagliptin was positively reflected on vascular responses of isolated aortic strips, heart rate and blood pressure values of the diabetic treated rats. In conclusion, the effectiveness and safety of sitagliptin for maintaining normoglycemic and normolipidemic levels. Along with its positive impact on antioxidant activity and vascular response during the treatment of diabetic rats, which could be considered as an additive value to DPP4- antagonists group.

Key words: Sitaglitin , STZ, HOMA, SOD and aortic strips.

INTRODUCTION

Diabetes is a common disease characterized by disturbance in blood sugar levels, metabolism of lipids, carbohydrates and proteins and increases the risk for cardiovascular diseases. The potential risk of type-2 diabetes (T2DM) may result in 75-90% of the excess risk of enhancing micro and macro vascular complications **(1)**. The development of T2DM was in accordance with the changes of insulin resistance, impaired glucose tolerance therefore, intensification of therapy is normally required over time **(2)**. Although the treatment of T2DM by current therapies of oral hypoglycemic agents represent major barriers to optimal glycemic control but not sufficient; because of their complex mechanism of actions or side effects such as

hypoglycaemia (sulphonylureas), obesity (thiazolidinediones), gastrointestinal intolerance (metformin), hypothyroidism, insulin resistance, atherosclerosis **(3,4)** .Oral antihypertensive and hypoglycemic drugs are widely used in many therapeutic areas. These drugs are effective and play an important role for controlling hypertention, heart failure and diabetes mellitus **(1)**.

Gliptins are a novel grade of oral anti-diabetic agent that enhances and prolongs the physiological actions of incretin hormones by competitively inhibiting the DPP-4 enzyme. Sitagliptin is DPP-4 enzyme antagonistic agent, which was approved for use in the USA in 2006 and launched in 2007, as the first member of a new class of oral hypoglycemic drug **(2)**. Sitagliptin is a specific type II diabetic drug, it can be used in combination with other anti-diabetic medications **(5)**.

Sitagliptin (Januvia) oral antidiabetic medicine has proven to be used as a suitable drug that helps in controlling blood sugar levels, increasing the insulin sensitivity and thereby reduce hyperinsulinemia. Sitagliptin controls elevated blood glucose levels through different ways, i.e., triggering pancreatic insulin secretion, regulation of the insulin level in the blood ,suppressing pancreatic glucagon secretion and directing the liver to reduce glucose production **(6,7)**.

Sitagliptin's mechanisms of action are thought to result from increased incretin levels [glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)], which inhibit glucagon release that increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels **(8)**. Sitagliptin is a highly selective Dpp-4 inhibitor for the treatment of T2DM that was discovered through the optimization class of β-amino acids derived DPP4 inhibitors. This action of DPP-4 inhibitor agoinst by potentiates the insulinotropic response to exogenous PACAP (pituitary adenylate cyclase activating peptide) and GRP (gastrin releasing peptide) in mice **(9)**.

Pharmacokinetics of sitagliptin is similar in healthy individuals and in T2DM patients. The absorption rate is (87%) and steady-state plasma concentration is reached after 3 days with terminal half-life of 10-12 hours at doses of 25-100 mg. sitagliptin is metabolized by cytochrome CYp3A4 and CYp2C8 and excretion are mainly through kidney **(10,11)**.

Therefore, the main objective of the current study was to evaluate the efficacy and protective performance of sitagliptin on vascular responses, antioxidant activity

as well as glucose and lipid metabolism of STZ-induce diabetic treated adult male rats.

Material and Methods:

- **Drugs and chemicals:**

1- Januvia^{R} (Sitagliptin 100 mg Tab.) manufactured by Merk sharp & Dahme (MSD), Italy. The daily dose of rat (10 mg /kg body weight).

2- Streptozotocin (STZ): rats of G2 &G3 were injected intra-peritonal once by 45 mg/kg B.wt. of STZ inducing experimental model of diabetes type II after 3 days.

- **Experimental animals:**

Thirty (30) male adult albino rats (weighing 150 ±10 gm) were used in the present study. They were obtained from the Animal House, Faculty of Medicine, Zagazig University. Animals were caged in separate cages, in controlled temperature (25^{o}C), humidity (60%), light and dark cycles of 12 hours each and allowed to acclimatize for two weeks. The animals were fed on standard pelleted diet and water *ad libitum*. The experiment was conducted in accordance with the ethical guidelines for investigation in the laboratory animals and was approved by the Ethical Committee of School of Medicine, Zagazig University, Egypt and complies with the Guide for the Care and Use of Laboratory Animals**.** The rats were divided into 3 equal groups each of 10 rats in separate units as the follows:

Group I: control rats received distilled water *ad labitum.*

Group II: diabetic control: STZ induce diabetic rats.

Group III: the diabetic rats treated with januvia^{R} (Stigliptin) at a dose of 10 mg/kg B.wt once a day orally for 30 successive days.

- **Induction of diabetes in animals and experimental design:**

After period of acclimatization, 20 of rats were injected intraperitoneally with streptozotocin (STZ) as a single dose of 45mg/kg body weight for induction of diabetes mellitus type II following the method of **Zafar et al. (12)**. Rats were considered diabetic when plasma glucose level was more than 289 mg/100 ml.

Sampling:

Blood samples were collected at the end of experimental period (after day 30th). The blood sample of 5 mL was collected from the carotid artery after scarification of rats into a centrifuge tube without anticoagulant, left to clot. Serum was separated and

centrifuged at 3000 rpm for 10 minutes. Clear serum was transferred carefully into clean and dry vials and kept frozen at -20 ºC until analyses for assaying serum glucose, insulin, lipid profile (Ch., TG, HDL and LDL), serum antioxidant enzyme (catalase) and serum malondialdehide (MDA). The aorta was obtained to evaluate the vascular relaxation after noradrenaline pre-contraction as well as the blood pressure and heart rate were measured for all studied groups.

Determination of glucose homoeostasis:

Glucose was determined according to the description of **Tietz (13)**. Serum insulin in rat was determined according to the method described by **Tample et al. (14)** using commercial Enzyme linked immunosorbent assay (ELISA) Kits**.** Homeostasis model assessment of insulin resistance (HOMA-IR) was measured as function of insulin resistance and β-cell function. The simplest and accurate equations was used as follows: HOMA-IR = insulin (µU/mL) x glucose (mg/dl) / 405 **(15).**

Determination of some antioxidant activities:

Serum catalase activity was determined according to the method of **Aebi (16)** by using Biodiagnostic kit method. Serum malondialdehyde activity (MDA) was determined according to the method of **Satoh (17)** by using Biodiagnostic kit method (Biodiagnostic Company, Dokki, Giza- Egypt).

Lipid profile assay:

Serum cholesterol levels were estimated according to the method of **Ellefson and Caraway (18)**; Triacylglycerols; HDL and LDL **(19)**.

Measurement of Blood Pressure and Electrocardiography:

Rats were anaesthetized by intraperitoneal (IP) injection of urethane (0.13g /100g body weight) and the blood pressure was estimated by by using pressure catheter coupled to a PowerLab data acquisition system & ECG were done by using cutaneous needle electrodes coupled also to the PowerLab data acquisition system in physiology Dep, Faculty of Medicine, Zagazig University.

Preparation of aorta for vascular response:

The isolated aorta of rats was cut into 10 × 1.0 mm helical strips and mounted on a myograph under a resting tension of 1.0 g in Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$,

and 5.7 mM glucose, pH 7.4) at 37°C and continuously bubbled with 5% CO_2 / 95% O_2 **(20)**. The aortic strips were initially vasoconstricted with 50 mM KCl, followed by a wash-out of the bathing medium; thereafter, a steady-state tension was obtained by using 1 mM noradrenaline. The obtained curves were recorded in all studied groups.

Statistical analysis

Data are expressed as the mean ± standard. Significant differences between the mean values of control, diabetic non-treared and sitagliptin-treated groups were evaluated using a one-way ANOVA. The difference was considered to be significant if the *P*-value was less than 0.05.

RESULTS:

Data presented showed a significant increase in the level of glucose compared to control one. However, there were no significant differences between the diabetic group treated with sitagliptin and the control one. The highest glucose level was recorded for diabetic group with an average value of 151.1 mg/dl. However, the glucose levels of control and diabetic group treated with sitagliptin were in normal values of 88.30 and 89.7 mg/dl, respectively **(Figure 1)**. The insulin levels revealed significant increase in their values in the control and sitagliptin treated diabetic rats. While, STZ induced diabetic rat groups showed a significant decrease in levels of insulin compared with other groups **(Figure 2)**.

In this study, we aimed to calculate insulin resistance (HOMA-IR) as function of insulin resistance and β-cell function. As mentioned above, the levels of glucose in diabetic rats was much higher than those of control and sitagliptin diabetic treated rats. In addition, the levels of insulin were much higher in control and sitagliptin diabetic treated rats to lesser extent of STZ induced diabetic rats. As a consequence, the calculated HOMA-Insulin resistant levels were significantly high in control and sitagliptin diabetic treated rats compared to the STZ induced diabetic rat **(Figure 3).**

Data presented in **Table (1)** shows the antioxidant activities of catalase and MDA. The obtained results showed a significant reduction of catalase activity in the diabetic rats compared to control and diabetic treated with sitagliptin. The levels of catalase activity of control and diabetic treatment treated with sitagliptin were almost 1.28 times the control treatment. However, MDA activity showed a different trend. The highest MDA activity was recorded for diabetic treatment with an average value of 113.20 mg/dl, which was 1.28 and 1.33 times the control and diabetic treated with

sitagliptin treatments, respectively. It seems that high blood-glucose level led to enhance the activity of MDA. Results from lipid profile revealed that the levels of cholesterol, triglyceride and low-density lipoprotein (LDL) were at a greater extent of diabetic treatment compared to control and diabetic treated with sitagliptin treatments. The high blood-glucose levels increased their levels in blood. The average blood cholesterol level of diabetic treatment was 2.4 and 2.3 times the control and diabetic treated with sitagliptin treatments, respectively. Similarly, the levels of triglyceride of diabetic treatment were 2.1 and 2.0 times the control and diabetic treated with sitagliptin treatments, respectively. The negative impact of high blood-glucose levels led to increase the level of low-density lipoprotein compounds. At the same time, the level of high-density lipoprotein level was significantly lower than those of diabetic treatment compared to other treatments.

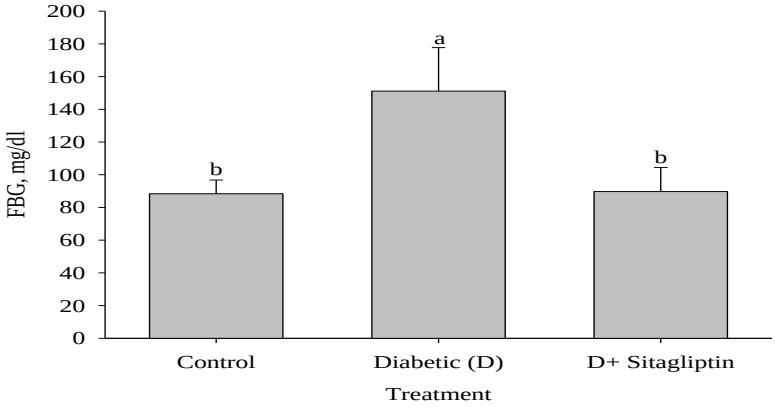

Figure (1) Fasting blood glucose (mg/dl) of all studied rats

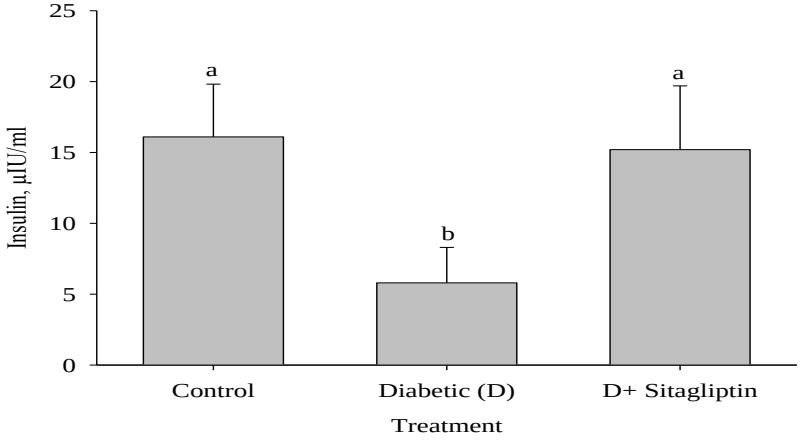

Figure (2) Insulin levels (µIU/ml) of all studied rats

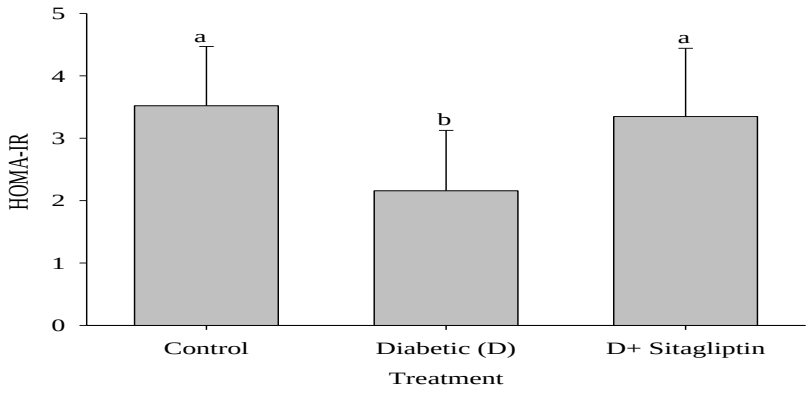

Figure (3): Calculated HOMA-Insulin resistant values of all studied rats

Table (1): Serum catalase enzyme, Malondialdhyde and lipid profile (mg/dl) in the studied groups:

Analysis	Control	Diabetic (D)	D + Sitagliptin
Catalase	790.70±17.21 [a]	619.0±72.48 [b]	794.20±12.23 [a]
MDA	88.16 ± 4.03 [b]	113.20± 20.57 [a]	85.39 ± 8.02 [b]
Cholesterol, mg/dl	72.2±14.71 [b]	176.2±16.47 [a]	77.2±11.82 [b]
Triglyceride, mg/dl	59.2±15.10 [b]	126.4±40.6 [a]	62.8±17.27 [b]
HDL, mg/dl	52.0±7.51 [a]	44.0±6.89 [b]	51.8±7.40 [a]
LDL, mg/dl	44.8±16.60 [b]	56.9±12.10 [a]	48.7±11.87 [b]

Values are expressed as mean±S.D (n=10). Means with different letter within row are statistically significant at (P< 0.05).

Data illustrated in **Figure (4)** shows the contractile vascular responses of isolated aortic strips (precontracted with NOR). Clearly in control treatment there is normal aortic relaxation **(Figure 4a)**. Similarly, in the diabetic-sitagliptin group showed a similar trend, normal relaxation was observed **(Figure 4c)**. However, In case of diabetic non treated group, a persistent aortic stimulation was observed **(Figure 4b).** Similar trend was observed in the blood pressure (mmHg) and ECG (mV) of the treated rats. The control and diabetic-sitagliptin treatments showed a regular blood pressure and ECG within normal conditions **(Figures 5 & 7)**. However, in case of diabetic non-treated group an asymmetrical routine of blood pressure and ECG levels was observed **(Figure 6).**

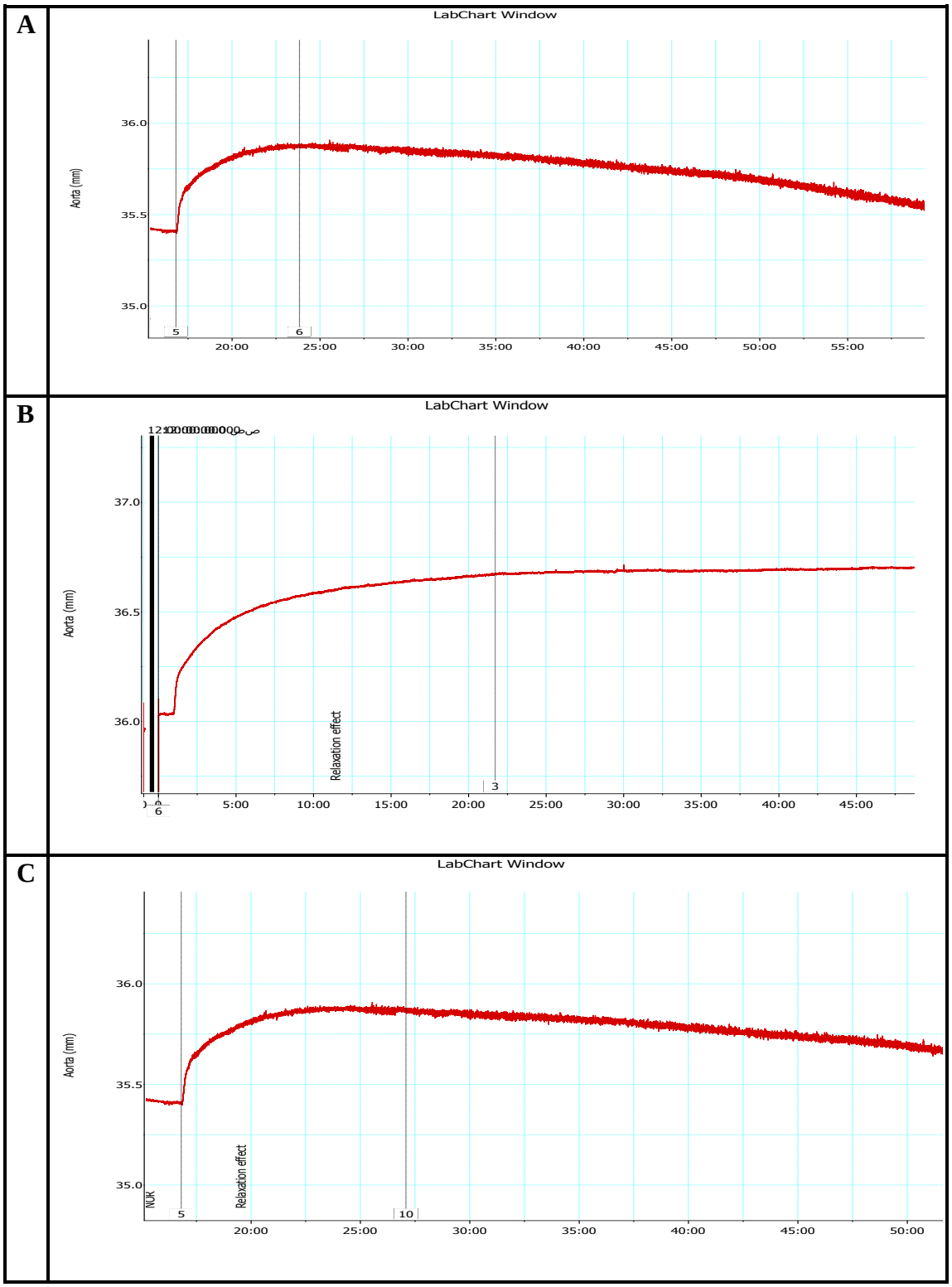

Figure (4): Tracing illustrating the contractile vascular responses of isolated aortic strips precontracted with NOR;(A) represented normal relaxation of control group, (B) represented a persistant contraction of diabetic non-treated group and (C) represented relaxation of diabetic group treated with sitagliptin.

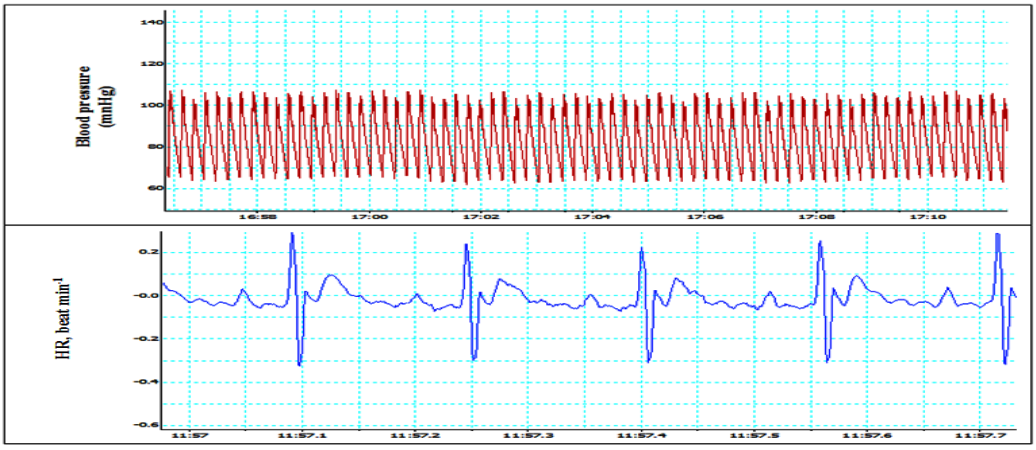

Figure (5): Tracing illustrating regular blood pressure (mmHg) and ECG (mV) for control group of rats

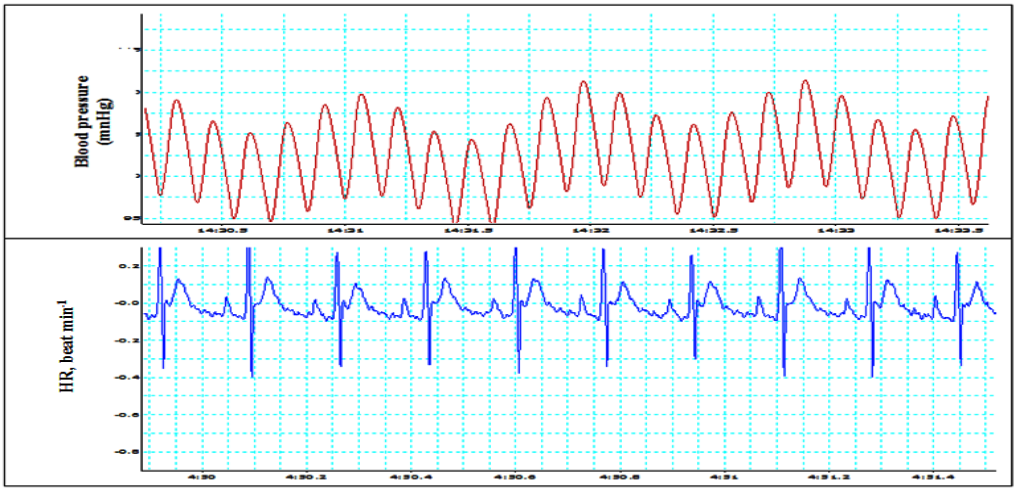

Figure (6): Tracing illustrating asymmetrical blood pressure (mmHg) and the ECG (mV) for STZ induce diabetes in the non-treated group

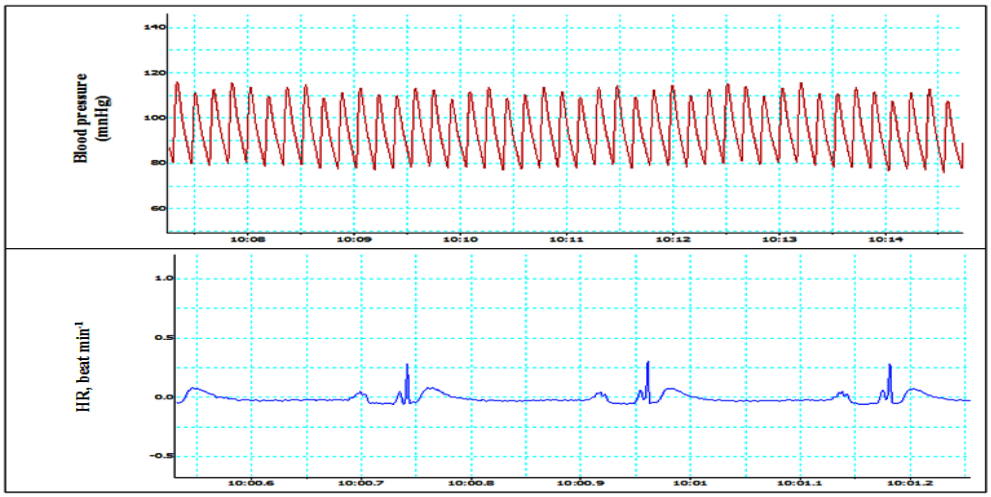

Figure (7): Tracing illustrating blood pressure (mmHg) and ECG (mV) for STZ induce diabetic rats treated with sitagliptin .

DISCUSSION

A major cardiac obstacle of diabetes is cardiomyopathy that develops independently of coronary artery disease, rise hypertension and valvular diseases, which may subsequently lead to heart failure and death **(21)**.

Dipeptidyl peptidase (DPP)-4 is a serine protease and cleaves X-proline dipeptides from the N-terminus of polypeptides **(20)**. DPP-4 inhibitors are the first new therapeutic class of oral antihyperglycaemic drug for T2DM for many years **(11)**. Identified mechanism of DPP-4 inhibitor mediated the blood glucose reduction after food intake while, DPP-4 is expressed also in numerous organs as kidney, liver, vascular tissues and heart **(22)**.

The aim of the current study was to evaluate the performance of sitagliptin on vascular and metabolic responses, as well as antioxidant activity among STZ-induce diabetic rats. The results of the present study indicated that the oral administration of sitagliptin for thirty successive days ameliorated the negative effects associated with diabetes type 2 in rats. Stiagliptin drug approved its efficacy in regulating the metabolic disturbance associated with diabetes type II.

Data presented showed that an increasing in the level of glucose with observable decreasing in the values of insulin hormone and HOMA-IR in STZ-induce diabetic rats. However, the use of sitagliptin in diabetic rats led to decrease the blood glucose levels as well as increased the insulin levels and HOMA-IR closed to the control (non-diabetic) treatment. These results are similar with the studies conducted by **Sharma et al. (23)** revealed a hypoglycemic effect of sitagliptin in STZ diabetic rats. **Alam et al. (24)** described that use of sitagliptin showed potential benefit in lowering increased blood glucose level in diabetes. **Davidson et al. (25)** concluded that sitagliptin enhance the body's own ability to control blood glucose by increasing the active levels of incretin hormones in the body.

Pospisilik et al. (26) went almost too similar findings, they have reported that Wister rats treated with streptozotocin twice-daily for 7 weeks exhibited lowered blood glucose, and increased levels of plasma insulin. Furthermore, improved glucose tolerance and increased pancreatic insulin content. Also, these results concur with **Mu et al. (27)** stated that, des-fluorositagliptin (DFS) significantly reduced blood glucose levels in diabetic mice; in addition it decreased plasma triglycerides and free fatty

acids. They have observed a profit for improvements in pancreatic insulin by increasing the β-cell mass and a reduction of the α-cell–to–β-cell ratio.

Additionally, **Ritchie and Abel (28)**; **Tate et al.(29)** confirmed that mark features of diabetes comprises hyperglycaemia, hyperinsulinaemia, insulin resistance, and renin-angiotensin-aldosterone system (RAAS) hyperactivation.

Sitagliptin (DPP-4 inhibitors) act by inhibiting the enzymatic degradation of glucagon-like peptide 1 (GLP-1) that delay gastric emptying, suppress glucagon release, and increase glucose-stimulated insulin release **(30).** Use of sitagliptin can increases GLP-1 levels and thereby decreases postprandial glucose excursions **(31).** As well, sitagliptin control elevated blood glucose by triggering pancreatic insulin secretion, suppressing pancreatic glucagon secretion, and signaling the liver to reduce glucose production **(8)** and they have attributed this action to the antidiabetic effect of sitagliptin.

For the effect of diabetes on lipid parameters, the obtained results showed that diabetic treatment without any medications led to increase the levels of serum cholesterol, triglyceride and LDL indicating the presence of hyperlipidaemia.. However, the use of sitagliptin enhanced these levels to normal conditions similar to the control treatment. Several studies demonstrated the effects of sitagliptin on lipid parameters in diabetic patients. They have observed that a significant reduction of TC ,trigluceride and LDL-C levels **(32)**, although anther study found that TC and LDL-C levels were unchanged by sitagliptin **(33)**.

Therefore, these results indicate that sitagliptin not only improves glycemic control, but also significantly reduces the elevated cholesterol, TG and LDL levels.

In this study, the STZ induced diabetic group showed an increased in the serum level of malondialadehyde (MDA) compared to control and sitagliptin treated groups. The high blood glucose levels may led to tissue damage, free radical production and eliminated the hydrolysis of lipid peroxides; consequently, increased levels of toxic aldehydes in rats was occurred to overcome these effects **(38).** The increased level of serum MDA in the STZ induce diabetic rats reflects tissue oxidative stress. **Ferreira et al. (35)** showed that in diabetic Zucker rats, the use of sitagliptin improved the oxidative stress.

In addition, there was an improvement in the activity of catalase enzyme after the use of sitagliptin in the diabetic rat compared to the STZ induce diabetic non-

treated rat. These results are in agreement with **Alam et al. (24)** explored the protective effect of sitagliptin by studying markers of oxidative stress. Previous study suggested that sitagliptin exerts anti-oxidative effects **(36)**.

The obtained data of vascular response on STZ induce diabetic rat showed a significant increase in the values of blood pressure and heart rates, The diabetic rat treated with sitagliptin provoked improvement in the cardiovascular activity representing normal blood pressure and heart rate parallel to the control group**.** These results go in accordance with **Shah et al. (37)** demonstrated that the DPP-4 inhibitor mediated vascular relaxation through a GLP-1–independent pathway. The circulating glucagon-like peptide (GLP)-1levels, which are emitted from the epithelium of the small intestine and have also been detected in vascular smooth muscle and endothelial cells **(38,39)**. Additionally, **Arakawa et al.(40)** reported that GLP-1 acts through GLP-1 receptors to prevent vascular endothelial cells from stress factors such as oxidative stress. **Takai et al. (20)** revealed that the inhibition of vascular DPP-4 activity by sitagliptin may contribute to its inhibitory effect on p22phox gene expression and MDA levels indicating an observed vascular protection. The vascular protective effect of sitagliptin may be dependent on the reduction of blood glucose levels.

The vascular reactivity to noradrenaline (NA) was tested on isolated rat aortic strips, the present study showed a significant increase in pressor response to NA in diabetic rats. This may be due to hyerglycemia induces endothelial dysfunction via the reduction of nitric oxide formation and the augmentation of ROS and contributes to the pathogenesis of vascular complications in diabetes or could be attributed to the presence of endothelial dysfunction and hypertension in diabetic rats **(41)**.

In contrast, the present study showed the treatment with stiagliptin in diabetic group of rats was significantly reduced the contractile response to NA and the same effect was obtained in the aortic strips curve of the control group. **Duvnjak and Blaslov (42)** reported that stiagliptin has a benficial role of stiagliptin glucose homostasis, blood pressure and artery function.

In brief, decreasing the risk of microvascular and macrovascular disease with respectable alterations in the major lipids can achieve by using antidiabetic medications as stitagliptin. Accordingly, this study enhances approach of drug selection for the better treatment of type 2 diabetes without diabetic complications.

CONCLUSION

Sitagliptin is one of DPP-4 antagonists having an effective action on the negative changes associated with STZ-induced diabetes mellitus in rats in term of improvement serum glucose, insulin levels and HOMA. On other hand, sitagliptin relief hyperlipidemia and increase the catalase enzymes reflecting on repairing the endothelial dysfunction and normalizes the vascular activity.

No Conflict of interest.

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