Cardio-Protective Effect of Vildagliptin and Rosuvastatin on Ischemia-Reperfusion Injury in Nicotinamide-Streptozotocin Induced Type 2 Diabetic Rats

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in individuals with diabetes, for which 65% of deaths are attributable to heart disease or stroke. This study was designed to evaluate the cardioprotective effects of vildagliptin and rosuvastatin alone and in combination on streptozotocin-induced type 2 diabetes (T2DM) in rats. Intraprotenial injection (IP) of nicotinamide (NA) 120 mg/Kg, followed by single IP administration of streptozotocin (STZ) 60 mg/Kg, was used for induction of T2DM in rats. Diabetic rats were divided into 4 groups. Diabetic-ischemic /reperfusion (I-R) non-treated group, Vildagliptin diabetic (I-R) pretreated group, Rosuvastatin diabetic (I-R) pretreated group and Vildagliptin + Rosuvastatin diabetic (I-R) pretreated group. Heart rate, ST segment elevation, Fasting blood glucose (FBG), Serum creatinine phosphokinase (CPK-MB), Troponin-I activity, Lipid profile, Serum Malondyaldehyde (MDA) and Serum Interluken-6 (IL-6) were measured. Both drugs showed significant improvement of electrophysiological and biochemical parameters which was confirmed with histopathological examination.

INTRODUCTION

Diabetes mellitus (DM) has been an important health problem in most nations with the number of patients dramatically soaring and expected to reach 366 million by the year 2030 worldwide[1].

DM is defined as a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin[2].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in individuals with diabetes, for which 65% of deaths are attributable to heart disease or stroke[3].

Most studies on cardiovascular risk in patients with diabetes have been performed in patients with T2DM, who are typically older at disease onset than those with type 1 diabetes[4]. Several studies have suggested that patients with T2DM without known coronary heart disease (CHD) have at least similar (if not higher) risk of cardiovascular mortality as patients without diabetes who had prior known CHD[5].

While the incidence of CHD has declined over time with improvements in risk factor modification, diabetes appears to persist as a significant risk factor for CHD[6]. Silent myocardial ischemia (SMI) is more frequent in diabetic patients leading to a delayed diagnosis and a more advanced stage of the CHD at the time of the diagnosis [7].

Dipeptidyl peptidase-4 (DPP-4) enzyme is a multifunctional protein that exerts biological activity through pleiotropic actions including: protease activity, association with adenosine deaminase (ADA), interaction with the extracellular matrix[8], cell surface co-receptor activity mediating viral entry and regulation of intracellular signal transduction coupled to control of cell migration and proliferation[9].

Physiological DPP-4 Substrates include glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and gastric inhibitory peptide or glucose-dependent insulinotropic polypeptide (GIP) while Pharmacological DPP-4 substrates include aprotinin, gastrin-releasing peptide, chronic gonadotropin and β-casomorphin[10].

Vildagliptin is a potent, selective and orally active 2nd generation DPP-4 inhibitors, with a reversible and competitive mechanism of action that binds and forms a complex with DPP-4

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enzyme, causing its inhibition. This results in improved glycemic control as determined by glycosylated hemoglobin (HbA1c) and fasting blood glucose (FBG) levels plus an enhancement of pancreatic α-and β-cell function[11].

Rosuvastatin is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Rosuvastatin has the most potent reduction of low density lipoprotein (LDL) and elevation of high-density lipoprotein (HDL) in this class[12].

Despite the fact that coronary heart disease is a complicated disorder associated with hypertension, hyperlipidemia, diabetes and heart failure, most experimental studies of cardio-protection depend on induction of heart injury in absence of other related diseases. Thus, we employed a combined diabetic-I-R rat model that is relevant to the clinical case of MI in diabetic patient.

The present was designed to explore the potential cardioprotective effect of vildagliptin and /or rosuvastatin on experimentally- induced I-R injury in type 2 diabetic rats model.

MATERIALS AND METHODS

Animals:

The experimental protocol met the national guideline on the proper care and use of animals in the laboratory research, Benha Scientific Research Ethics Committee approved the study.

Adult male albino rats (n=50), each 150-170 g. at the beginning of the study. They were purchased from Faculty of Veterinary medicine, Benha University, Al Qalyubiah, Egypt. All animals were housed. They have acclimatized for one week in controlled laboratory condition at 20 -25°C in a 12h light\dark cycle and had free access to standard diet and water.

Drugs:

Vildagliptin (powder), **Rosuvastatin** (powder), other chemicals and reagents (Sigma-Aldrich co., Cairo. Egypt.)

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Experimental protocol:

Animal grouping:

Rats were divided into 5 groups, 10 rats each as follow:

Group (I): Sham -operated normal control group.

The rats of this group were injected with single IP injection of 0.6 ml of citrated buffer then underwent the same procedure without ligation of LAD coronary artery[13].

Group (2): Diabetic (I-R) non treated group.

Diabetic rats were not given any medication prior to induction of (I-R)[14] after development of NC-STZ type 2 DM.

Group (3): Vildagliptin pretreated diabetic (I-R) group.

Diabetic rats were given vildagliptin 6mg /kg/day orally for 4 weeks[15] prior to induction of (I-R) after development of NC- STZ type 2 DM.

Group (4): Rosuvastatin pretreated diabetic (I-R) group.

Diabetic rats were given rosuvastatin 1 mg/kg/day orally for 4 weeks[16] prior to induction of (I-R) after development of NC- STZ type 2 DM.

Group (5): Vildagliptin and Rosuvastatin pretreated diabetic (I-R) group.

Blood samples were collected from right carotid artery in clean and dry centrifuge tubes, which were left for 15 min to clot and then centrifuged at 3000 rpm to separate the serum and stored at -20^0C for biochemical analysis.

Experimental induction of NA-STZ type 2 DM:

Experimental diabetes was induced by a single intraperitoneal (I.P) injection of STZ 60 mg/kg, freshly dissolved in cold citrate buffer, pH 4.5 after 15 min of I.P injection of NA 120 mg/kg prepared in normal saline[17]. Hyperglycemia was confirmed by the elevated levels of random blood glucose which determined at 72 h. then on day 7 after injection. Only rats confirmed to have type 2 DM were used in the study. The animals

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with fasting blood glucose concentration more than 250 mg/dl were used for the study[18].

Experimental induction of ischemia-reperfusion (I-R) injury in NA-STZ diabetic rats [13]:

a- Diabetic rats were anesthetized with urethane in a dose of 1.5- 1.75 gm/kg body weight. Half of the dose was injected IP, to induce rapid onset and the other half subcutaneously, to insure long maintenance of the anesthetic effect. The body temperature was monitored and maintained at 37°C throughout the experimental protocol. After complete anesthesia, the rats were led on their back. The neck was opened with a ventral midline incision, tracheostomy was performed and the rats were ventilated with room air from a positive pressure ventilator (Inco, India) using compressed air at a rate of 70 strokes /min and a tidal volume of 10 ml/kg. A left thoracotomy was performed at the fifth intercostal space and the pericardium was opened to expose the heart. The proximal left anterior descending (LAD) coronary artery was identified and transiently tied with a slipknot 4–5 mm from its origin for a 30- minute ischemic period, but without exteriorization of the heart. To allow cardiac reperfusion, by releasing the slipknot, after the completion of the surgical procedure, the heart was returned to its normal position in the thorax. The diabetic rats then underwent 30 min ischemia and 4 hours reperfusion but in sham control rats, the procedure is identical, except the LAD is not transiently ligated[13].

b- ECG records were done using needle electrodes. The four limb electrodes were fixed to the animal's four limbs and records were done using the standard lead II at rate of 25m/min, E.C.G. tracings were recorded during ligation, immediately, 30 min, 1, 2 and 4 hours after reperfusion. Occlusion was confirmed visually *in situ* by the appearance of a whitish pallor of the left ventricle (LV), appearance of regional epicardial cyanosis and ST-segment elevation[19]. The myocardium was reperfused by releasing the snare gently for a period of 4 hours. Successful reperfusion was confirmed by visualization of arterial blood flow through the artery and appearance of hyperemia over the surface of the previously ischemia cyanotic segment [13].

Parameters measured:

Electrophysiological parameter: Determination of

a- ST segment changes.

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b- Heart rate (HR) by ECG.

Biochemical measurements:

1- Fasting blood glucose (FBG) [20].

2- Serum creatinine phosphokinase (CPK-MB) level[21].

3- Troponin-I activity[22].

4- Serum lipoproteins (lipid profile):

a- Total cholesterol level[23].

b- Triglycerides level[24].

c- LDL-C and HDL-C level[25].

5 –Oxidative stress and inflammatory marker.

a- Serum Malondialdehyde (MDA)[26]

b- Serum Interluken-6 (IL-6)[27].

Histopathological examination:

After functional studies were completed, the heart was excised as a whole, put on cold (8°C) 30 mM KCl to achieve diastolic arrest[28]. Both atria were excised; the ventricles were preserved in neutral formalin 10% and referred for histopathological examination. The hearts were cut into transverse sections from the ventricle and interventricular septum, each section was fixed with methanol and ethanol (1:1), processed with paraffin wax, sectioned at 5μm and stained with hematoxylin and eosin. The cardiac sections were examined under light microscopy for the presence of myocyte degenerative changes[29].

Statistical analysis:

Results were presented as mean ± standard deviation (mean ± SD). Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) to detect significant differences between the group means. Tukey Kramer post-test was used to determine level of significance. Probability (P) values of < 0.05 were considered as statistically significant[30].

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RESULTS

Heart rate (HR) and ST segment elevation changes:

In diabetic (I-R) non treated group there was statistically significant increase (p<0.05) in HR and ST segment elevation compared to control group. Pretreatment of diabetic (I-R) group with VIL resulted in significant decrease (p<0.05) in ST segment elevation compared to diabetic (I-R) non treated group but it was still at significant higher level (p<0.05) if compared to control group. Pretreatment of diabetic (I-R) group with ROS resulted in significant decrease (p<0.05) in HR and ST segment elevation compared to diabetic (I-R) non treated group but it was still at significant higher level (p<0.05) if compared to control group. While (VIL + ROS) treated diabetic (I-R) group resulted in significant decrease in HR and ST segment elevation (p<0.05) compared to diabetic (I-R) non treated group with more significant effect (p<0.05) if compared to each drug alone (Table 1, Figure 1, 2, 3, 4, 5).

Table (1): HR and ST segment elevation in different studied groups (Mean ± SD):

Parameters Groups	Heart rate (beat/min)	ST segment elevation (mm)
Normal control group	280 ± 20	0
Diabetic (I-R) **non treated group**	407 ±35[a]	8.4 ± 0.83[a]
	↑45.4%*	↑>100%*
VIL pretreated diabetic (I-R) **group**	390 ± 31[a]	5.4 ± 0.62[a,b]
	↓4.3%**	↓35.7%**
ROS pretreated diabetic (I-R) **group.**	350 ± 30[a,b,c]	5.3±0.68[a,b]
	↓14%**	↓37%**
VIL+ROS pretreated diabetic (I-R) **group**	320 ± 25[a,b,c,d]	2.85 ±0.32[a,b,c,d]
	↓21.4%**	↓66.1%**

a: Significant difference versus Normal control group at p<0.05.

b: Significant difference versus diabetic (I-R) - non treated group at p<0.05.

c: Significant difference versus VIL treated diabetic(I-R) group at p<0.05.

d: Significant difference versus ROS treated diabetic (I-R) group at p<0.05.

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N.B:

* % change is calculated in relation to control group.

** % change is calculated in relation to diabetic untreated group.

Fasting blood glucose (FBG) level changes:

In diabetic (I-R) non treated group there was significant increase (p<0.001) in FBG level compared to control group. Pretreatment of diabetic (I-R) group with VIL or ROS resulted in significant reduction (P < 0.001) & (P<0.05) respectively in FBG level compared to diabetic (I-R) non-treated group. While in combination (VIL+ROS) treated diabetic (I-R) group, there was significant reduction of FBG level (P<0.05) & (P< 0.01) respectively compared to VIL treated and ROS treated diabetic (I-R) group (Table 2).

Serum level of CPK-MB and Troponin-I changes:

In diabetic (I-R) non treated group there was significant increase (p<0.05) in CPK-MB fraction and serum troponin-I level compared to control group. Pretreatment of diabetic (I-R) group with VIL or ROS resulted in significant reduction (p<0.05) in CPK-MB fraction and serum troponin-I level compared to diabetic (I-R) non treated group, but it was still at significant higher level (p<0.05) if compared to control group. While in combination (VIL+ROS) treated diabetic (I-R) group resulted in significant decrease in CPK-MB fraction and serum troponin-I level (p<0.05) compared to VIL treated and ROS treated diabetic (I-R) groups, but it was still at significant higher level (p<0.05) if compared to control group (Table 2).

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Table (2): FBG, serum CK-MB and serum Troponin-I in different studied groups (Mean ± SD):

Parameters	FBG(mg/dl)	CK-MB (U/L)	Troponin-I (ng/ml)
Normal control group	110 ±5.7	564±45.5	0.38±0.02
Diabetic(I-R) **non treated group**	286±13.1[a]	1532±100.4[a]	3.45±0.32[a]
	↑>100%*	↑>100%*	↑>100%*
VIL pretreated diabetic(I-R) **group**	160±9.5[a,b]	970 ±83.6[a,b]	2.25±0.17[a,b]
	↓44.1%**	↓36.7%**	↓34.8%**
ROS .pretreated diabetic (I-R) **group.**	230±8.9[a,b]	890 ±68.8[a,b]	1.23±0.09[a,b,c]
	↓19.6%**	↓41.9%**	↓64.3%**
VIL+ ROS treated diabetic (I-R) **group**	125±6.7[b,c,d]	624±55.4[a,b,c]	0.9±0.05[a,b,c,d]
	↓56.3%**	↓59.3%**	↓73.3%**

a: Significant difference versus Normal control group at p<0.05.

b: Significant difference versus diabetic (I-R) non treated group at p<0.05.

c: Significant difference versus VIL pretreated diabetic(I-R) group at p<0.05.

d: Significant difference versus ROS pretreated diabetic(I-R) group at p<0.05.

N.B:

* % change is calculated in relation to control group.

** % change is calculated in relation to diabetic untreated group.

Lipid profile changes:

In diabetic (I-R) non treated group there was significant increase (p<0.05) in total serum cholesterol, serum triglycerides and LDL-C levels with significant decrease (p<0.05) of HDL-C level compared to control group. The present study demonstrated a significant improvement (p<0.05) in these parameters in ROS treated and in (VIL+ROS) treated diabetic (I-R) group (p<0.05) compared to diabetic (I-R) non-treated and VIL treated

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groups, but it was still at significant higher level (p<0.05) if compared to control group (Table 3).

Table (3): Lipid profile in different studied groups (Mean ± SD)

Parameters Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Normal control group.	103±8.4	100±82	36.5±2.6	39±2.7
Diabetic (I-R) non-treated group.	215±14.6[a]	210±14.2[a]	18.5±2.1[a]	120±9.4[a]
	>100%*	>100%*	↓49.3%*	>100%*
VIL pretreated diabetic (I-R) group.	185±4.1[a]	172±4.3[a,b]	20.4±2.1[a]	97.5±7.8[a,b]
	↓14 %**	↓18 %**	↑10.3%**	↓ 18.8%**
ROS pretreated diabetic (I-R) group. diabetic	137±10.2[a,b,c]	134±9.8[a,b,c]	26±2.4[a,b,c]	68.9±6.7[a,b,c]
	↓35.3 %**	↓36.2 %**	↑45.9%**	↓42.6%**
VIL+ROS pretreated diabetic (I-R) group.	125.5±9.2 [a,b,c]	123±8.9[a,b,c]	32±2.8 [a,b,c,d]	52.5±6.3[a,b,c,d]
	↓ 41.9%**	↓ 41.4%**	↑73%**	↓56.3 %**

a: Significant difference versus Normal control group at p<0.05.

b: Significant difference versus diabetic(I-R) non treated group at p<0.05.

c: Significant difference versus VIL pretreated diabetic(I-R) group at p<0.05.

d: Significant difference versus ROS pretreated diabetic(I-R) group at p<0.05.

N.B:

* % change is calculated in relation to control group.

** % change is calculated in relation to diabetic untreated group.

Serum Levels of (MDA) and (IL6) in different studied groups:

MDA and IL6 was significantly (p<0.05) increased in diabetic (I-R) non treated group compared to control group, their levels significantly (p<0.05) decreased in VIL and ROS

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pretreated groups, both groups showed insignificant difference between them, but still at significant higher level (p<0.05) compared to control group. Co- administration of (VIL+ROS) significantly (p<0.05) decreased (MDA) and (IL6) levels compared to monotherapy groups and untreated group but (MDA) still at significant higher level (p<0.05) compared to control group.

Table (4): Serum levels of (MDA) and (IL6) in different studied groups (Mean ± SD).

Groups	Serum (MDA) nmol/L	Serum (IL6) nmol/L
Control group	22.1± 2. 2	0.284±0.02
Untreated diabetic (I-R) group.	92.5 ±9.1[a]	1.408±0.15 [a]
	↑ >100% *	↑ >100% *
VIL pretreated group	60.2± 5. 3[a,b]	0. 787±0.07 [a,b]
	↓34.9%**	↓44.1%**
ROS pretreated group	56.8 ±5. 2[a,b]	0.752±0.06 [a,b]
	↓38.6%**	↓46.6%**
VIL+ROS pretreated group	39.1± 4. 4[a,b,c,d]	0.314 ±0.029 [b,c,d]
	↓57.7%**	↓77.7%**

a: Significant difference versus Normal control group at p<0.05.

b: Significant difference versus diabetic (I-R) non treated group at p<0.05.

c: Significant difference versus VIL pretreated diabetic (I-R) group at p<0.05.

d: Significant difference versus ROS pretreated diabetic (I-R) c group at p<0.05.

N.B:

* % change is calculated in relation to control group.

** % change is calculated in relation to diabetic untreated group.

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Histopathological examination:

Histopathological examination of the heart for detection of signs of acute ischemia and inflammation was done at the end of the experiment in different groups:

Group (1) control normal group: showed normal cardiac muscle with central elongated nuclei, eosinophilic cytoplasm and striations without any infraction or infiltration of inflammatory cells (Fig.6).

Group (2) Diabetic (I-R) group with no medication: showed marked degenerative changes in the form of (cardiac muscle necrosis with congested blood vessels, hemorrhage and interstitial edema) (Fig.7).

Group (3&4) Diabetic (I-R) VIL or ROS pretreated group: showed moderate degeneration of cardiac muscle, congested blood vessels and interstitial edema (Fig. 8, 9).

Group (5) Diabetic (I-R) VIL + ROS pretreated group: showed mild degenerative changes in the form of mild (cardiac muscle necrosis, congested blood vessels and interstitial edema (Fig.10).

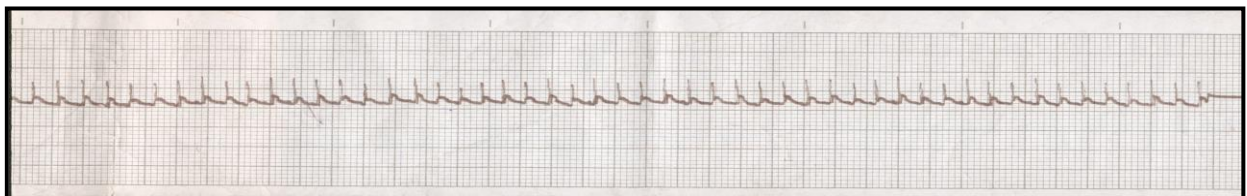**Fig. (1): ECG Tracing (Lead II) of control normal rat.**

Fig (2): ECG Tracing (lead II) of diabetic (I-R) non treated rats.

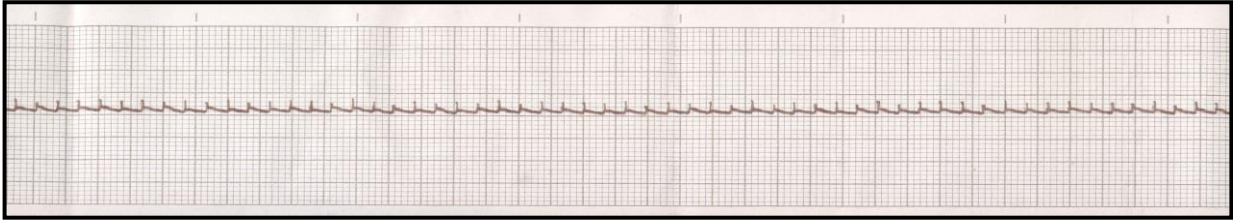*Citation: Hanan T. Emam et al. Ijppr.Human, 2019; Vol. 15 (4): 22-45.*

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Fig (3): ECG Tracing (lead II) of vildagliptin diabetic (I-R) pretreated rats.

Fig (4): ECG Tracing (lead II) of rosuvastatin diabetic (I-R) pretreated rats.

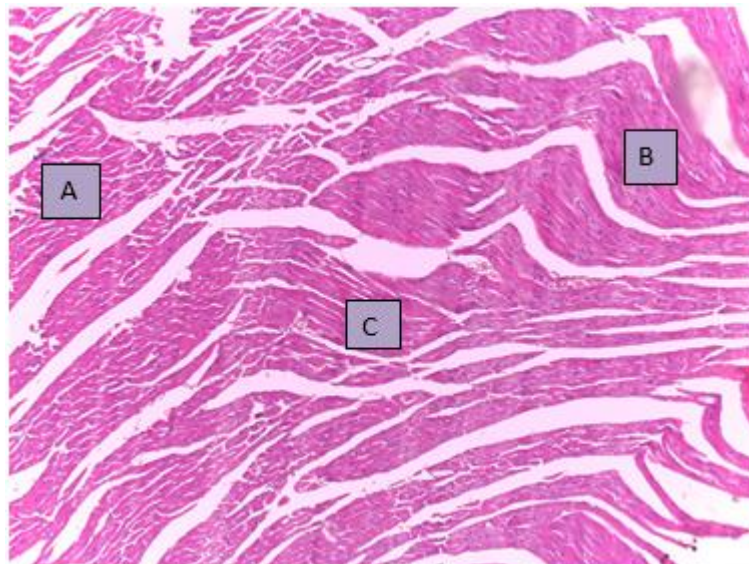**Fig (5): ECG Tracing (lead II) of vildagliptin+ rosuvastatin diabetic (I-R) pretreated rats.**

Fig (6): Cut section of the heart showing normal cardiac muscle with (A) elongated nuclei and (B) eosinophilic cytoplasm with (C) striations (H & E × 200).

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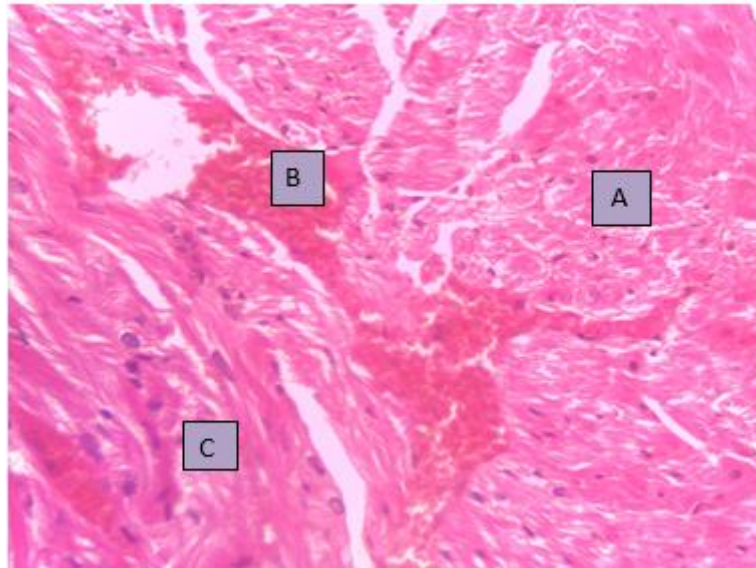
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Fig (7): Cut section in the heart of diabetic (I-R) rat (group 2) showing (A) Cardiac muscle necrosis, (B) Congested blood vessels, hemorrhage (C) Interstitial edema (H&Ex400).
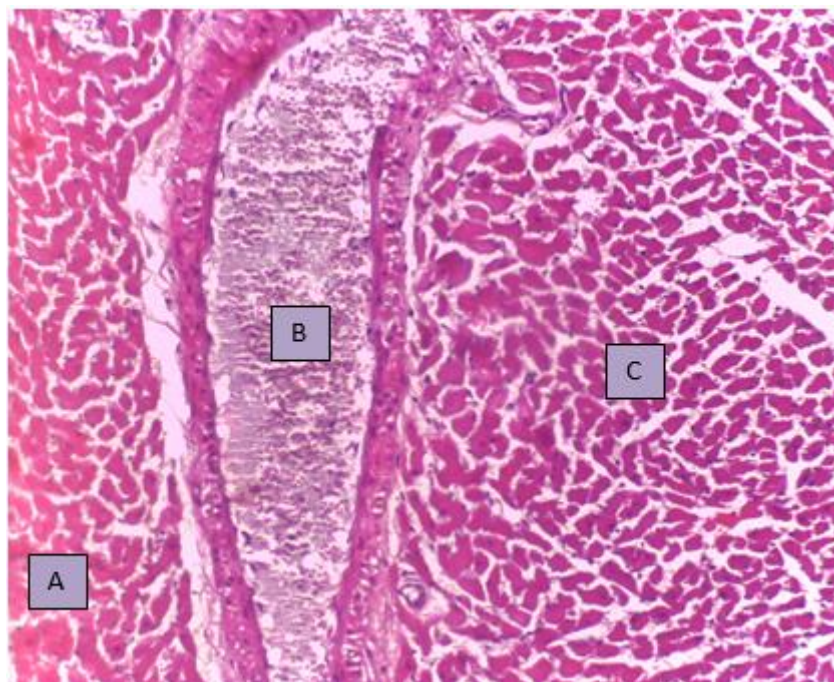

Fig (8): Cut section in the heart of vildagliptin diabetic (I-R) pretreated rat (group 3) showing (A) Moderate cardiac muscle necrosis. (B) congested blood vessel. (C) Interstitial edema (H&Ex400).

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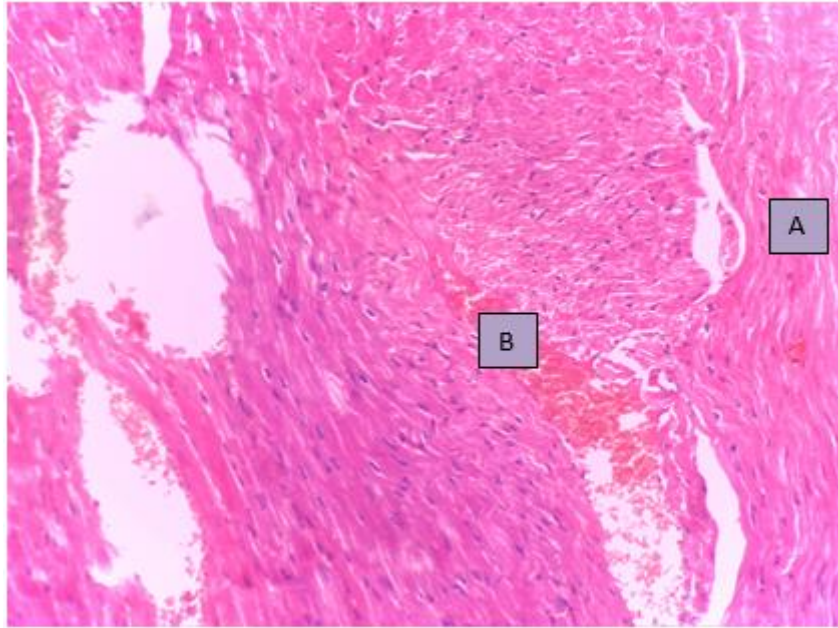
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Fig (9): Cut section in the heart of rosuvastatin diabetic (I-R) pretreated rat (group 4) showing (A) moderate cardiac muscle necrosis. (B) congested blood vessel. (H&Ex200).
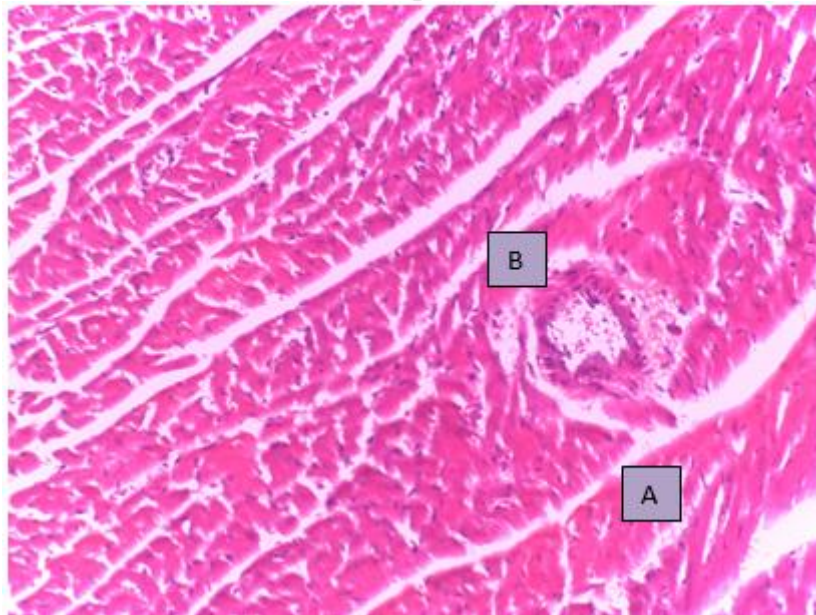

Fig (10): Cut section in the heart of vildagliptin+ rosuvastatin diabetic (I-R) pretreated rat (group 5) showing (A) Mild cardiac muscle necrosis. (B) Mild congested blood vessel. (H&Ex400).

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DISCUSSION

Diabetes mellitus (DM) is currently a major health problem all over the world and is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors[31].

T2DM is one of the leading causes of morbidity and mortality due its complication like nephropathy and other cardiovascular diseases, accounting for at least 10 % of total health care expenditure in many countries[32].

The obtained data in the current work revealed that induction of T2DM of experimental rats by NA-STZ resulted in hyperglycemia at 72 h which was confirmed by the elevated blood glucose level, on day 7 after injection, only rats confirmed to have permanent T2DM with blood glucose concentration more than 250mg/dl were used in the study[17,18].

STZ is an antibiotic used to induce experimental diabetes in animals[33]. STZ-induced diabetes may be due to glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety, mainly O at 6 position of guanine. The transfer of methyl group from STZ to the DNA molecule causes damage which results in fragmentation of DNA and functional defects of the beta cells. Moreover, STZ has potential to act as an intracellular nitric oxide (NO) donor and generates ROS. The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ[34].

Therefor STZ - induces diabetes by free radical generation, which causes a massive reduction of insulin secreting beta cells of the islets of Langerhans, resulting in a decrease in endogenous insulin release[18].

The anti diabetogenic effect of nicotinamide may be due, in part, to an increase in the pool size of NAD++ in beta-cells. NAD++ is the principal metabolite of nicotinamide. It appears that the pool size of NAD++ in beta- cells in pre-diabetics and diabetics is significantly reduced. Damage and destruction of beta-cells may occur via oxidative stress. Increased levels of ROS in beta-cells may result in, among other things, oxidative damage to DNA resulting in strand breaks[18]. The poly ADP ribose polymerase or PARP is believed to play a role in DNA repair. PARP uses NAD++ as its substrate. In the context of a reduced level of NAD++, PARP activity may essentially use most of the cellular NAD++[35]. This could

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result in cellular apoptosis. Nicotinamide is an inhibitor of PARP. It also has antioxidant activity and, of course, is metabolized to NAD++. All of these effects may play some role in the possible anti diabetogenic action of nicotinamide. Nicotinamide by the above mechanism opposes the effects of STZ, helps in partial destruction of beta cells and helps in the development of T2DM[36].

In the present work induction of I-R by ischemia for 30 min and reperfusion for 4 hours in these experimentally NA-STZ type 2 diabetic rats resulted in significant elevation of serum CPK-MB level and serum troponin-1 level[37]. Also resulted in significant elevation of HR and ST segment elevation [14].

Vildagliptin is one of members of (DPP-4s) inhibitors which commonly called gliptins that enhances islet cell insulin secretion via an augmented incretin effect, has a high affinity for DPP-4 enzyme that improves glycemic control. [38].

The data of the present work showed that, administration of vildagliptin 6mg /kg / day orally as monotherapy for 4 week prior to induction of I-R in the experimentally NA-STZ type 2 diabetic rats resulted in significant reduction of FBG level, serum CPK-MB level, serum troponin-1 level and ST segment elevation with non- significant changes of HR compared to diabetic I-R non treated rats.

These data are in agreement with, **Burkey *et al*., (2005)** and **Liu *et al*., (2012)** [39,40] who reported that pretreatment of insulin-resistant and diabetic rats with vildagliptin significantly improved glycemic and lipid profile.

Also, these data are in line with **Chinda *et al.,(*2014)**[14] who reported that administration of single dose of vildagliptin IV as monotherapy 30 min prior to induction of AMI by (I-R) injuries in the experimental rats resulted in non- significant changes of HR and significant reduction of ST segment elevation and infarcted size.

The result can be explained by **Mitani *et al*., (2002)**[41] who mentioned that action of vildagliptin was accompanied by a marked increase in the glucose-stimulated levels of intact GLP-1, enhanced glucose-stimulated insulin levels, and a marked decrease in glucose excursions after an oral glucose challenge.

Cardio-protective effect of vildagliptin in this study can be explained by **Chinda *et***

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al.,(2014)[14] who mentioned that vildagliptin has protective effect on cardiac mitochondria during I/R. Mitochondria also play pivotal roles in myocardial survival or death following I/R. The burst of ROS level during I/R period caused mitochondrial permeability transition pore (mPTP) opening, resulting in dissipating of adenosine triphosphate (ATP) and mitochondrial swelling, which subsequently lead to an activation of the apoptotic pathway and eventually cardiac cell death[42].

Also, vildagliptin alleviated myocardial apoptosis by increasing anti-apoptotic protein Bcl-2 causing the less activated caspase3, thus resulting in higher level of pro-caspase3. [14]

Statins (HMG-CoA) reductase inhibitors are the mainstay of lipid lowering drug therapy in patients with hyperlipidemia. Since patients with NAFLD are at high risk to develop cardiovascular disease (CVD), statins are frequently prescribed to patients with NAFLD and hyperlipidemia [43]. Statins are commonly used in the treatment of hypercholesterolemia and coronary artery disease (CAD)[44].

Rosuvastatin is a lipid-lowering agent that competitively inhibits the HMG-CoA reductase enzyme, rosuvastatin exhibits the highest efficacy in the reduction of LDL cholesterol, total cholesterol and triglycerides compared with other statins, it has been shown that rosuvastatin has an increased number of binding sites to the HMG-CoA reductase enzyme compared with other statins, which would explain its stronger inhibition capability and thus its greater therapeutic efficacy[45].

The present study showed that pretreatment with rosuvastatin orally as monotherapy 1mg /kg/day for four weeks before induction of I-R significantly reduced heart rate and ST segment elevation. These data are supported by previous studies[46] which revealed that statins improve myocardial functions and improve histopathological picture of the heart in rats.

On the other hand, our findings have demonstrated that pretreatment of diabetic rats with rosuvastatin for 4 weeks before I-R significantly lowered the leakage of the cardiac biomarkers (CPK-MB level). These data are supported by previous studies[47] which revealed that statins improve myocardial functions and concluded that such observations could be attributed to the potential role of rosuvastatin in protecting the myocardial membranes and in alleviating the extent of myocardial damage.

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The present study showed that pretreatment of diabetic rats with rosuvastatin 4 weeks before I-R significantly reduced total cholesterol, triglycerides, LDL and significantly increased HDL. The obtained data are in harmony with previous studies **(Cheng, 2004)[48]** who concluded that rosuvastatin significantly reduced total cholesterol, triglycerides, LDL and significantly increased HDL.

These results are also in agreement with (**Kipshdize and Kapanadze, 2008)[49]** who revealed that rosuvastatin treatment was significantly reduced plasma concentrations of total cholesterol, triglycerides, low density lipoproteins and increase HDL level.

These results are also in line with (**Koksal *et al*., 2011)[50]** who demonstrate that rosuvastatin treatment was noted to reduce plasma concentrations of total cholesterol, triglycerides and LDL in hypercholesterolemic patients. Hypocholesterolemic effect is mainly mediated via HMG-CoA reductase inhibition [51].

In addition, the histopathological changes of pretreated groups showed retardation of inflammation and myonecrosis which could be attributed also to the antioxidant and anti-inflammatory properties of rosuvastatin. These data are supported by previous studies [47,52] which revealed that statins improve myocardial functions and improve histopathological picture of the heart.

Moreover, these data are also supported by previous studies **(Dourado *et al*., 2011)[46]** who revealed that rosuvastatin prevents myocardial necrosis, improved myocardial functions and improved histopathological picture of the heart in rats in an experimental model of acute myocardial infarction.

Despite the molecular biological activities of statins, which clearly show anti-inflammatory and antioxidant effects, the results of randomized trial evaluating rosuvastatin failed to show a reduction in cardiovascular events [53].

The exact mechanisms by which rosuvastatin prevent myocardial necrosis are beyond the scope of our study. But it has been established by the work of others. According to **(Davignon and Laaksonen, 1999) [54];** statins have several effects related to the reduction of LDL-C levels. However, this class of drugs has other metabolic manifestations such as pleiotropic effects that are independent of cholesterol reduction. The fundamental question that remains is whether these effects promote additional cardioprotection than the known

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LDL-C-reducing action[55]; have suggested four non-lipidic mechanisms that may contribute to the beneficial effects on clinical events, these effects include modification of endothelial function, inflammatory response, plaque stabilization, and thrombus formation. Hypocholesterolemic effect of rosuvastatin is mainly mediated via HMG-CoA reductase inhibition [51], rosuvastatin is also known to had pleiotropic effects, which seem to be independent of their lipid-lowering action, such as anti-atherosclerotic, anti-inflammatory, anti-thrombosis, and anti-oxidant effects, these properties could potentially reduce the necrotic area in the setting of AMI[56].

Furthermore, several studies demonstrated that ROS suppressed inflammatory responses through inhibition of nuclear factor-kappa B in endothelial cells and protected rodents from ischemic stroke and myocardial reperfusion injury, suggesting that rosuvastatin-mediated benefits are dependent on its anti-oxidant and anti-inflammatory activity in various types of cells[57].

Rosuvastatin, a lipid-lowering agent in clinical practice, has an appreciable anti-atherogenic property which is due to the improvement of endothelial dysfunction as well as its anti-thrombotic, anti-inflammatory and antioxidant effects[47], it has been reported to prevent MI induced experimentally , it possesses a number of biological activities such as antioxidant, antihyperlipidemic, anti-inflammatory and hepatoprotective effects, statins improve endothelial function and decrease plasma levels of tumor necrosis factor-α) (TNF-α) in patients with coronary artery disease and hyperlipidemia[58].

Statins have been considered to prevent atherosclerosis by way of improvement of endothelial function, modulation of inflammatory responses and inhibition of thrombus formation. Statins are understood to increase NO bioavailability by either 1) directly activating nitric oxide synthase (NOS) through phosphorylation,[59] 2) improving NOS mRNA stability by increasing mRNA half-life and subsequent transcription[60] and 3) stimulating the production of tetrahydrobiopterin[61], an important NOS cofactor required for NO production. This increase in NO affords important vascular and hemodynamic benefits capable of mediating many of statins pleiotropic effects [56].

Several previous studies demonstrated the beneficial cardio-protective effect of combination therapy between vildagliptin and other anti-diabetic drugs as metformin [15] and

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dapagliflozin[62]**,** but -up to our knowledge – no study demonstrate the combination between vildagliptin and rosuvastatin.

According to the present study, co-administration of vildagliptin + rosuvastatin as a pretreatment of diabetic rats for 4 weeks before I-R significantly improved HR, cardiac biomarker, FBG level, lipid profile, MDA and IL6 compared with monotherapy either with vildagliptin or rosuvastatin ,these results were confirmed with histopathological examination, this greater cardioprotective effect may be due to different mechanisms of both drugs .

In conclusion, vildagliptin and rosuvastatin may have cardio-protective effect in diabetic patient, such finding may suggest the use of these drugs in diabetic patient particularly if these patients prone to or have any ischemic heart diseases.

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