

Myocardial salvaging effects and mechanisms of dipeptidyl peptidase-IV inhibitor Vildagliptin in experimental diabetes

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Abstract

Background: Morbidity, mortality and re-infarction rate are higher following myocardial infarction in diabetics than non-diabetic subjects. Recently, glucagon-like peptide (GLP)-1 was shown to have cardioprotective effects, but treatment with GLP-1 is limited by its short half-life. It is rapidly degraded by the enzyme dipeptidyl peptidase-IV (DPP-IV). We hypothesized that DPP-IV inhibitor Vildagliptin will increase levels of GLP-1 and may confer cardioprotective effects in setting of diabetes beyond its effect on glycemia.

Methods: Diabetes was induced with single dose of Streptozotocin (STZ): 45mg/kg ip and myocardial infarction was produced by administering isoproterenol (ISP): (85mg/kg, sc) to rats 24 and 48 h prior to sacrifice (5th week). After the confirmation of diabetes on 7th day (Glucose \geq 200mg/dl), Vildagliptin (10 mg/kg) was administered and various parameters like anti-diabetic (Glucose, HbA1c), cardioprotective (CPK-MB, hs-CRP), hypolipidemic (lipid profile, atherogenic potential), antioxidant (MDA) safety {pancreatic function (lipase), liver function (SGPT), kidney function (Creatinine)} and histopathological indices of injury were evaluated in experimental groups.

Results: Vildagliptin (10 mg/kg) treatment demonstrated significant antidiabetic as well as myocardial salvaging effects as indicated by restoration of blood glucose, HbA1c and CPK-MB levels compared to Diabetic- ISP Control group. In addition, Vildagliptin favorably modulated the lipid parameters (TC, TG, HDL-C, LDL-C), atherogenic index, lipid peroxidation (MDA). Subsequent to ISP challenge, histopathological assessment of heart, pancreas and biochemical indices of injury confirmed the cardioprotective effects of Vildagliptin in setting of diabetes.

Conclusion: The present study concluded that Vildagliptin treatment demonstrated myocardial salvaging effects in type II diabetic rats challenged with experimental Myocardial infarction.

Keywords: Vildagliptin, Myocardial Infarction, Diabetes, Streptozotocin, Isoproterenol.

1. Introduction

The Diabetes mellitus is a common risk factor for coronary artery disease with a higher incidence of myocardial infarction and sudden death.[1] Morbidity and mortality are higher in myocardial infarction in diabetic than non-diabetic subjects.[2,3] Although antihyperglycemic drugs improve glycemic control, the study by United Kingdom Prospective Diabetes, has found that metformin treatment may contribute to cardiovascular benefits to certain subgroups of patients. While similar beneficial effects found by pioglitazone in Proactive study.[4,5] However, there is still not sufficient evidence that antidiabetic therapies reduce the cardiovascular risk of patients with diabetes.

GLP-1 analogues have been used for the treatment of type II diabetes because of its multiple actions on pancreatic function.[6] Besides its effects on glucose

metabolism, lot of clinical and experimental study suggested that GLP-1 exert cardiovascular effects in the presence or absence of diabetes.[7] Administration of GLP-1 improves myocardial function and cardiac output in experimental models of cardiac injury or heart failure. However, in vivo half-life of GLP-1 is very short because it is degraded by dipeptidyl peptidase –IV enzyme, An alternative approach for enhancing GLP-1 action involves the use of DPP-IV inhibitors.[8] The various experimental studies evaluated the protective effect of DPP-IV inhibitors in cardiovascular disease in hypertension, heart failure, and myocardial infarction.[9-13] Recently it was reported that the DPP-IV inhibitor Vildagliptin may exert beneficial effects on infarcted hearts by inhibiting the degradation of active GLP-1 and other cardiovascular peptides also Vildagliptin has favorable effect

on cardiac hypertrophy and diastolic dysfunction in rats.[14] Despite the above mentioned studies on cardiac function, the effects of DPP-IV inhibition Vildagliptin on experimentally induced myocardial infarction in setting of type II diabetic rats has not been studied.

The purpose of this study was to investigate potential cardioprotective effects and mechanisms of Vildagliptin subsequent to isoproterenol induced myocardial infarction in the setting of diabetes mellitus on various components of viz. anti-diabetic (blood glucose, HbA1c), hypolipidemic (lipid profile, atherogenic index). In addition to understand the underlying mechanisms; anti-inflammatory (hs-CRP), antioxidant (MDA), cardioprotection (CPK-MB) and also the safety parameter {pancreas [lipase (U/L)], liver [SGPT (U/L)], renal [creatinine (mg/dl)]} function and histopathological indices of injury were evaluated.

2. Material and Methods

2.1 Experimental Animals

Adult male Wistar rats, 10 to 12 weeks old, weighing 150 to 200 gm were used in the study. Rats were housed in the Animal Facility of Mahatma Gandhi Mission Medical College, Navi Mumbai, India in polyacrylic cages (38x23x10cm) under standard laboratory conditions. The study protocol was approved by the Institutional Animal Ethics Committee and conforms to the *Committee for the Purpose of Control and Supervision of Experiments on Animals* and Indian National Science Academy and Guidelines for the Use and Care of Experimental Animals in Research. The animals were allowed free excess to standard diet, tap water *ad libitum* and allowed to acclimatize for two weeks before the experiments.

2.2 Chemicals and drugs:

Streptozotocin (STZ) and Isoproterenol (ISP) were procured from Sigma Chemicals St Louis, USA. The test drug Vildagliptin was obtained as gift sample. All other chemicals and reagents used were of analytical grade.

2.3 Experimentally induced myocardial infarction in setting of Type II diabetes mellitus:

Male Wistar rats weighing 150- 200 gm was used for the study. Type II Diabetes was induced in rats by a single STZ injection (45 mg/kg body wt, i.p. dissolved in 0.01 M citrate buffer, pH 4.5) in overnight fasting rats. Serum glucose estimations (blood sugar \geq 200 mg/dl) were undertaken periodically (day 0, 3, from the tail vein) to confirm the production of diabetes mellitus. Animals showing fasting blood glucose higher than 200 mg/dL were considered as diabetic and used for the further study. Myocardial infarction was produced by Isoproterenol (85mg/kg) sc injection 24 and 48 hr prior to scarification (5th weeks). At the end of experimental period, rats were sacrificed, Blood sample were collected for further biochemical investigation and histopathological evaluation.

2.4 Experimental Groups

Group 1: Normal Control (NC): In Normal Control group, rats were administered distilled water per orally using a feeding cannula for study period of 5 weeks.

Group 2: Diabetic ISP Control (D-ISP): The Streptozotocin (45 mg/kg body wt, i.p. dissolved in 0.01 M citrate buffer, pH 4.5) was injected ip to induce diabetes at 0 week and challenged with Isoproterenol (85 mg/kg body wt sc dissolved in saline) 24 and 48 h prior to scarification (5th week).

Group 3: Vildagliptin (VIL): Vildagliptin (10 mg/kg) was fed orally from 1st to 5th week (4 weeks). The Streptozotocin (45 mg/kg body wt, i.p. dissolved in 0.01 M citrate buffer, pH 4.5) was injected ip to induce diabetes at 0 week. Subsequently the rats were challenged with Isoproterenol (85mg/kg body wt sc dissolved in saline) 24 and 48 h prior to scarification (5th week).

2.5 Evaluation parameters:

2.5.1 Assessment of body weight changes:

Each rat was weighed individually twice, first at the beginning of the experiment (initial weight) and second, 24h after the administration of the last dose of either drug (final weight). The difference in body weight of each rat was calculated and expressed as percentage change according to the following:

% change in body weight =

$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

2.5.2 Biochemical Parameters

The blood samples of all the experimental rats of different groups were collected from the retro-orbital plexus under light anesthesia at 0, 1, 3 and 5 weeks for estimation of blood glucose, CPK-MB. In addition, after the completion of the experimental duration (5th week), serum was used for the determination of the following parameters like lipid profile, pancreatic lipase, SGPT, creatinine, hs-CRP by Auto-analyzer or ELISA kits in the Pathology (NABL accredited) and Pharmacology laboratory. MDA was estimated using k. Satoh's method.

2.5.3 Histopathological studies

At the end of the experiment, the animals were sacrificed. The heart and pancreas were immediately fixed in 10% buffered neutral formalin solution. The tissues was carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross sections (5 μ m thick) of the fixed tissues were cut. These sections were stained with hematoxyline and eosin and visualized under light microscope to study the microscopic architecture of the tissues. The investigator performing the histological evaluation was blind to biochemical results and to treatment allocation.

3. Results

3.1 General observations and Assessment of body weight changes:

Diabetic rats, in general, showed classical symptoms of overt diabetes, with signs such as polydipsia, polyphagia

and polyurea and after receiving ISP were beset with, within minutes, extremely rapid respiration. The severity of the symptoms became prominent myocardial infarction in setting of Type II diabetes mellitus. The D-ISP group rats showed significant ($p < 0.001$) decrease in body weight (%) as compared with NC. VIL (10 mg/kg) treatment showed significant ($p < 0.05$) restoration in body weight as compared with D-ISP group.

3.2 Diabetic Parameter:

Blood glucose was measured with one touch glucose meter. There was a significant ($p < 0.001$) increase in blood glucose in D-ISP group rats as compared to NC group rats till 5 weeks. Oral feeding of VIL (10 mg/kg) significantly restored ($p < 0.001$) the elevated blood glucose levels. Similarly glycosylated hemoglobin was also reduced in VIL (10 mg/kg) treatment group as compared to the D-ISP rats at 5 weeks.

3.2 Cardiac parameter:

There was a significant ($P < 0.001$) increase in serum CPK-MB level in D-ISP rats as compared to NC group. Treatment with VIL (10 mg/kg) significantly ($P < 0.001$) reduced elevated serum CPK-MB levels as compared to D-ISP group subsequent to ISP challenge. The other cardiac markers hs-CRP was found to be significantly reduced ($p < 0.05$) in VIL (10 mg/kg) as compared with D-ISP group at 5th week of study after ISP challenge.

3.3 Lipid Parameter:

The D-ISP group rats showed significant ($p < 0.001$) increase in serum TC, TG, LDL and decrease in HDL when compared with NC group of rats. VIL (10 mg/kg) treatment significantly ($p < 0.001$) reduced the levels of TC, TG, LDL and increased HDL levels as compared with D-ISP group rats at 5th week. D-ISP rats displayed the highest level of atherogenic index and were found to be significantly different ($p < 0.05$) from the NC group. The administration of VIL to diabetic rats after challenge with ISP significantly decreased ($p < 0.05$) these indices as compared with D-ISP group.

3.4 Oxidative stress parameter:

The D-ISP group rats showed a significant ($p < 0.01$) increase in the level of Oxidative marker (MDA) when compared to NC group rats at 5th weeks. The administration of VIL to diabetic rats subsequent to challenge with ISP significantly decreased MDA levels ($p < 0.05$) as compared with D-ISP group.

3.5 Safety Parameter:

The pancreatic, liver and kidney marker enzymes were analyzed in the different experimental groups. The D-ISP group rats showed a significant ($p < 0.01$) increase in the level of Lipase (U/L), SGPT (U/L) and creatinine (mg/dl) when compared to NC group rats at 5th weeks. The VIL treated group did not adversely affect the pancreas, liver and kidney function markers as compared with D-ISP group rats at 5th weeks.

Fig 1: The % change in body weight of NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. *** $p < 0.001$ NC Vs D-ISP, ^s $P < 0.05$ D-ISP Vs VIL.

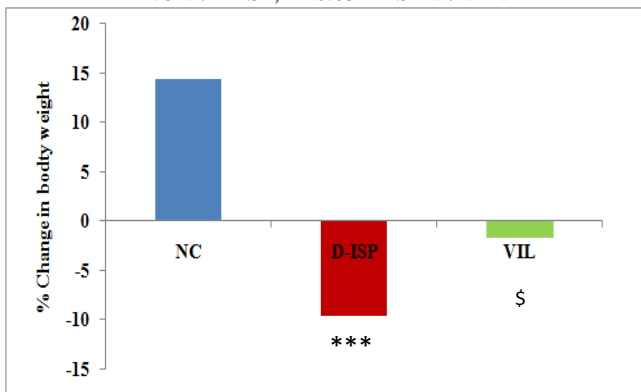


Fig 3: The HbA1c level of NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. *** $p < 0.001$ NC Vs D-ISP, ^{\$\$\$} $P < 0.001$ D-ISP Vs VIL.

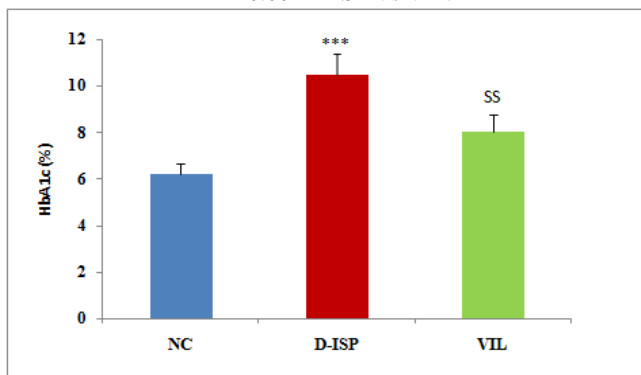


Fig 2: Time course changes of Blood Glucose level of NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. *** $p < 0.001$ NC Vs D-ISP, ^{\$\$\$} $P < 0.001$ D-ISP Vs VIL.

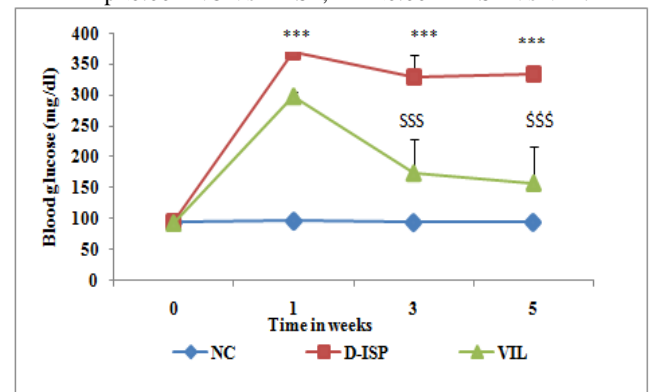


Fig 4: The CPK-MB of NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. *** $p < 0.001$ NC Vs D-ISP, ^{\$\$\$} $P < 0.001$ D-ISP Vs VIL.

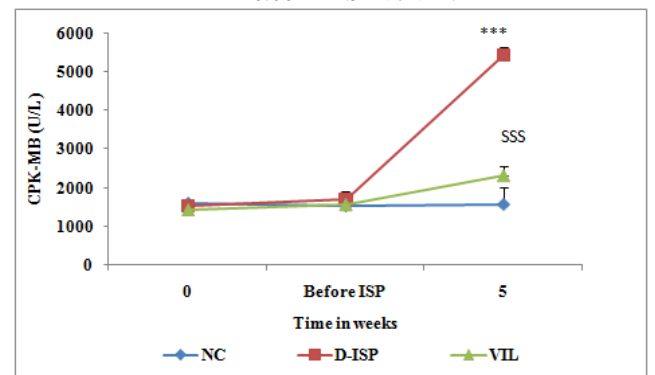


Table 1: Lipid profile in various experimental Groups

SN	Variable	NC	D-ISP	VIL
1	TC (mg/dl)	67.5±5.08	84.14±3.71 ^{**}	77.3±9.37 [§]
2	TG (mg/dl)	74.14±4.87	89.71±3.94 ^{**}	92.62±5.33 [§]
3	HDL (mg/dl)	32.66±2.66	23.57±3.63 ^{**}	30.87±3.22 [§]
4	LDL (mg/dl)	14.83 ±0.97	17.94± 0.78 ^{**}	18.52 ±1.04 [§]

NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. ^{**}p<0.01 ^{*}p<0.05 NC Vs D-ISP, [§]P<0.01 D-ISP Vs VIL.

Table 2: Study variables in the experimental groups

SN	Variables	NC	D-ISP	VIL
1	Cardiac Variables Hs-CRP (mg/dl)	0.83±0.15	1.9±0.5 ^{**}	0.91±0.1 [§]
2	Oxidative Marker MDA (nmol/ml)	1.84±0.07	4.25±0.18 ^{**}	3.23±0.46 [§]
3	Pancreatic Marker Lipase(U/L)	33.33±2.19	40.13±5.82 ^{**}	38.53±3.62
4	Liver function SGPT(U/L)	64.12±2.91	84.54±2.10 ^{**}	74.36±8.68 [§]
5	Kidney function Creatinine(mg/dl)	0.32±0.06	0.42±0.03 ^{**}	0.48±0.07 [§]

NC (n=8), D-ISP group (n=7), VIL (n=7). Values are expressed as mean±SD. ^{**}p<0.01 NC Vs D-ISP, [§]P<0.05 D-ISP Vs VIL.

3.6 Histopathological assessment

a) Plate 1: A-C: Histopathology of the Heart:

Microscopic histology revealed that the NC group was characterized by an organized pattern and shows normal architecture of the myocardium (Plate 1A). However, in the rat sections of the D-ISP control group, myonecrosis with fibroblastic proliferation, infiltration of inflammatory cells, marked intramyocellular edema, congested blood vessels as compared to NC group was observed (Plate 1B). VIL treatment prevented myonecrosis, infiltration of inflammatory cells, edema and vacuolar changes as compared to the D-ISP control (Plate 1C). (H&E x 40)

b) Plate 2: A-C: Histopathology of the Pancreas:

Microscopic histology revealed that the NC group (Plate 2A) rats were characterized by an organized pattern and showed normal architecture of islets of langerhans and the beta cells. In contrast, the rat sections of the D-ISP control group demonstrated damaged islets of langerhans, atrophy of beta cells and reduced beta cell mass as compared to NC (Plate 2B). Pancreas of rats from VIL treatment showed improved beta cell mass, less inflammatory infiltration and hemorrhage as compared to D-ISP group (Plate 2C). (H&E x 40)

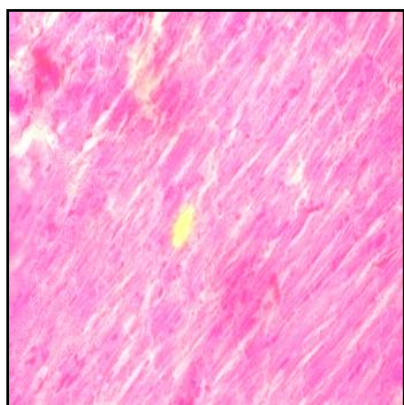
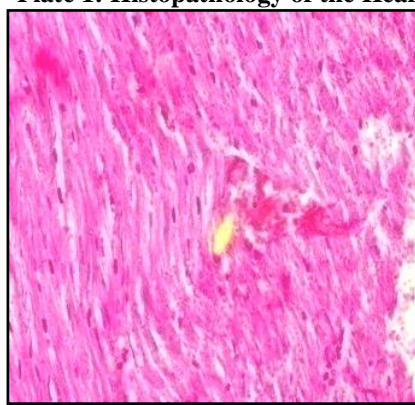
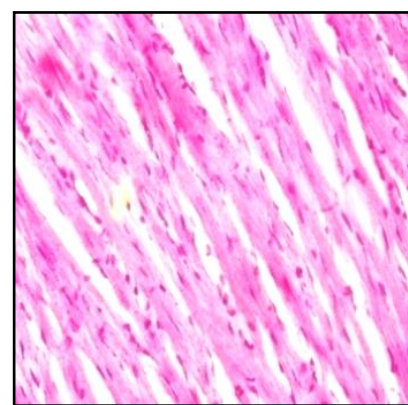
Plate 1: Histopathology of the Heart**1A: Normal control****1B: Diabetic ISP control****1C: Vildagliptin**

Plate 1: Heart: 1A: NC group rat heart revealed the non-infracted architecture of the myocardium. **1B:** D-ISP Control group rats subjected to STZ and ISP injury, demonstrated marked edema, confluent areas of necrosis and separation of myofibers, congested blood vessels and inflammation. **1C:** Vil treatment group rats, occasional focal myofiber loss, inflammation, necrosis and edema was observed. However the degree of edema, inflammation and necrosis was less as compared to the Diabetic-ISP Control group. (H&E x 40)

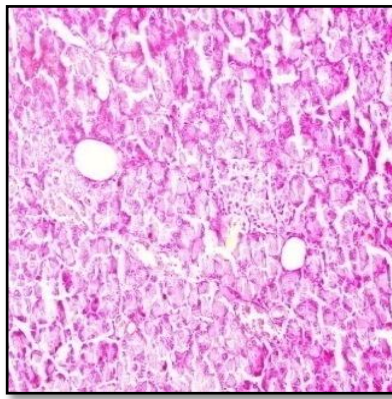
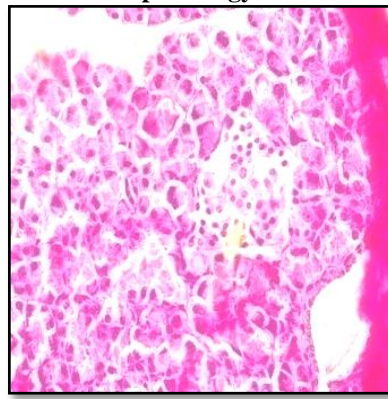
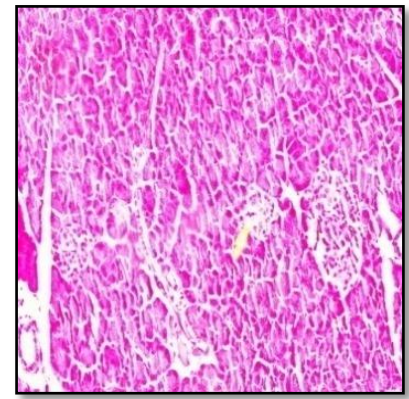
Plate 2: Histopathology of the Pancreas**2A: Normal control****2B: Diabetic ISP control****2C: Vildagliptin**

Plate 2: Pancreas: **2A:** NC rats pancreas were characterized by an organized pattern and showed normal architecture of islets of Langerhans and the beta cells. **2B:** D-ISP Control group of rats demonstrated damaged islets of Langerhans, atrophy of beta cells and reduced beta cell mass. **2C:** VIL treatment group rats Pancreas shows, improved beta cell mass, less inflammatory infiltration and hemorrhage as compared to Diabetic-ISP group. (H&E x 40)

4. Discussion:

DPP-IV Inhibitors are a novel class of anti-diabetic drugs that are widely being used clinically. DPP-IV Inhibitor like Vildagliptin has multiple beneficial effects reported in isolated studies like anti-diabetic, cardioprotective, anti-inflammatory and antioxidant. However, there is no experimental evidence presently available with regard to the possible beneficial effects of Vildagliptin on attenuating changes observed in myocardial infarction co-existing with diabetes in experimental rats. This is the first report of the efficacy of Vildagliptin in experimental model of myocardial infarction in the setting of diabetes.

The significant finding of this study is that Vildagliptin ameliorates myocardial infarction co-existing with diabetes induced deleterious changes in experimental rats. Vildagliptin treatment favorably modulated diabetes (Blood glucose, HbA1c), hypolipidemic (favorable lipid profile, atherogenic index), cardioprotective (CPK-MB) parameters in the experimental model of diabetes with myocardial infarction. Also to understand the mechanisms; anti-inflammatory (hs-CRP levels), antioxidant (MDA) and safety parameters {pancreas [lipase (U/L)], liver [SGPT (U/L)], renal [creatinine (mg/dl)]} contributing to the beneficial effects of Vildagliptin in diabetes with myocardial infarction was studied.

4.1 Essential components of myocardial infarction co-existing with diabetes:

4.1.1 Anthropometric parameter:

% change in body weight was evaluated in the NC, D-ISP and VIL groups. The % change in body weight; 14.45 % in NC, -10.03% in D-ISP and -1.78 % in VIL treatment group respectively. Various clinical and experimental study found similar results, Vildagliptin did not show significant effects on weight gain. [15]

4.1.2 Diabetes:

In the present study, the D-ISP rats showed significant increase in blood glucose, glycosylated hemoglobin which was ameliorated after administration of Vildagliptin at the end of 5th weeks. Shamim *et al* [16], Kakadiya, *et al* [17] and Rajesh Kumar *et al* [18] showed increase levels of blood glucose, glycosylated hemoglobin in D-ISP rats similar to present study. A significant decrease was observed in the glucose and HbA1c levels in diabetic-ISP rats after treatment with Vildagliptin when compared with D-ISP rats at the end of experimental period. Akarte *et al* [19] supported the antihyperglycemic effects of vildagliptin in STZ induced rats. The antidiabetic activity of vildagliptin may be improved insulin secretion and peripheral insulin sensitivity. The DPP-IV inhibitor vildagliptin is a novel class of antidiabetic medication. Inhibition of DPP-IV by vildagliptin prevents degradation of GLP-1 and reduces glycemia in patients with type II diabetes mellitus, with a low risk for hypoglycemia and no weight gain. Vildagliptin binds covalently to the catalytic site of DPP-IV, eliciting prolonged enzyme inhibition. This raises intact GLP-1 levels, both after meal ingestion and in the fasting state, thereby producing hypoglycemic effects.

4.1.3 Dyslipidemia:

The role of dyslipidemia in the development of diabetes macrovascular complications is well known. In our study, the STZ-ISP-model of diabetes exhibited abnormalities in lipid metabolism as evidenced from the significant elevation of serum TC, TG, LDL-C and reduction of HDL-C levels. Study by Sanna Khan *et al* (2015) showed treatment with Vildagliptin significantly reduced the TC, TG, LDL-C level and increased HDL-C levels in diabetes rats. [20] The Vildagliptin treatment also showed favorable effects on atherogenic index.

4.1.4 Cardiac variable:

The abnormal high levels of CKP-MB is claimed to be a specific and extremely sensitive index of myocardial necrosis or ischemia. The present study determined the CPK-MB levels to confirm the myocardial injury induced by STZ-ISP in rats. However, treatment with Vildagliptin significantly restored increase in serum CPK-MB levels at 5th week. Vildagliptin is an antidiabetic agent which exerts its beneficial effects in the cardiovascular system through glycometabolic control. This finding are in agreement with an earlier study reporting that significantly reduced serum CPK-MB levels in diabetes with myocardial infarction rats.[18] The Myocardial injury induced by STZ-ISP shown by biochemical marker was also confirmed by histopathological assessment.

4.4.5 Mechanism; inflammatory, oxidant Variables:

Inflammatory mediators, such as hs-CRP are increased in diabetes and inflammation seems to play a key role in the pathogenesis of myocardial dysfunction. The present data is in accordance with Hadi NR, *et al* (2013) demonstrated that Vildagliptin treatment significantly reduces the elevation of inflammatory markers (hs-CRP) in atherosclerosis model of hypercholesterolemia suggesting that vildagliptin inhibit vascular inflammation induced by high atherogenic diet.[21] The most commonly used indicator of lipid peroxidation is TBARS. In the present study, TBARS was increased significantly in D-ISP group rats. TBARS, a marker of oxidative stress, was reduced with vildagliptin treatment, suggesting ability of this drug in regulating oxidative stress. Our data are in accordance with the previous report by Maeda S *et al* [22].

4.4.6 Safety variable:

Pancreatic Lipase was assessed to detect degree of pancreatic injury. Increased Lipase levels as seen in D-ISP rats showed presence of pancreatic tissue damage as compared to NC. The pancreatic lipase was not significantly altered in Vildagliptin treatment group as compared with D-ISP. Histopathology of pancreas showed restoration in the architecture of the pancreas.

In the present study D-ISP treated rats showed increased levels of SGPT enzyme. Numerous studies have reported that diabetes is associated with raised levels of SGPT a marker of hepatic injury; In addition, recent evidence suggests that diabetes is associated with changes in morphology and eventually functional alteration in kidneys. In keeping with the potential mechanisms through which DPP-IV inhibition may exert a nephroprotective effect, Liu *et al* have recently reported that vildagliptin attenuates renal injury in streptozotocin-induced diabetic rat. [23] The present results clearly demonstrate raised liver and kidney function marker in serum of D-ISP rats. In contrast, the Vildagliptin treated rats showed significant reduction in these markers, thus showing its ability to protect against STZ/ISP induced damage.

Thus, the beneficial effects of Vildagliptin (10 mg/kg) as shown by present study reveals its protective effects on deleterious changes induced by myocardial infarction co-existing with diabetes via multiple mechanisms: hypoglycemic, hypolipidemic, antioxidant, cardioprotective and anti-inflammatory property.

5. Conclusion:

The present study concluded that Vildagliptin at 10 mg/kg demonstrated myocardial salvaging effects in type II diabetic rats challenged with experimental Myocardial infarction. There may be several mechanisms (favourable effects on lipid profile, reduced atherogenic potential, anti-inflammatory, antioxidant) contributing to the cardioprotective effects of Vildagliptin.

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