

Mitigating effects of vildagliptin in experimental diabetes with metabolic syndrome

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ABSTRACT

Background: Vildagliptin has multiple beneficial effects reported in isolated studies like anti-diabetic, cardio protective, 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 metabolic syndrome co-existing with diabetes in experimental rats. Thus, the present study was designed to evaluate potential effects of vildagliptin on various components of metabolic syndrome. Also to elucidate the underlying mechanisms: DPP-IV, anti-inflammatory, antioxidant pathways were studied.

Methods: A combination of high fat diet (HFD) and low dose of streptozotocin (STZ) 40 mg/kg was used to induce metabolic syndrome co-existing with diabetes mellitus in wistar rats. The HFD were fed to rats for 10 weeks to induce metabolic syndrome. At the end of 3 weeks, diabetes was induced by a single STZ injection (40 mg/kg body weight). Vildagliptin (10 mg/kg) was administered to rat from 5th to 10th weeks daily and various parameters of Diabetes and metabolic syndrome were studied. Also to understand the mechanisms; DPP-IV pathway, anti-inflammatory, antioxidant parameters were studied. Biochemical indices of injury (pancreatic, liver and renal function) and histopathological assessment of injury was evaluated in experimental groups. Immunohistochemistry of pancreas was done to assess beta cell mass.

Results: The vildagliptin treatment ameliorated the deleterious effects associated with metabolic syndrome and diabetes. The beneficial effects demonstrated by vildagliptin on various parameters include: anti-diabetic (reduced blood glucose, HbA1c, HOMA-IR, increased serum insulin, HOMA-β and restoration of pancreatic function), central obesity (reduced body weight, abdominal circumference (AC), thoracic circumference (TC), AC/TC ratio) and hypolipidemic (favourable lipid profile, atherogenic index) activity. A significant restoration of cardiac injury as indicated by CPK-MB levels was observed. In addition, DPP-IV pathway (reduced serum DPP-IV), anti-inflammatory (reduced hs-CRP levels), and antioxidant (reduced MDA) contributed its beneficial effects in diabetes with metabolic syndrome model. The protective effects on heart, pancreas, liver and kidney were confirmed by histopathological report. The immunohistochemical report of pancreas showed preservation of beta cell mass in vildagliptin treated rats.

Conclusions: Vildagliptin treatment ameliorates deleterious changes of diabetes with metabolic syndrome. Beneficial effects of vildagliptin can be attributed to hypoglycemic, hypolipidemic, antioxidant, cardioprotective and anti-inflammatory effects.

Keywords: Vildagliptin, Diabetes, Metabolic syndrome, HFD, STZ

INTRODUCTION

The metabolic syndrome represents a cluster of abnormalities, including obesity, insulin resistance, dyslipidaemia and type II diabetes, which increases the risk of developing cardiovascular diseases. The metabolic syndrome is a clinically and socially important issue which has drawn the attention of many physicians and researchers. Studies have demonstrated that patients with metabolic syndrome displayed an increased risk of developing diabetes, cardiovascular disease and other diseases.¹

The incretin hormone, glucagon-like peptide-1 (GLP-1), has multiple metabolic effects that would be desirable attributes of an oral antidiabetic agent.² These include glucose-dependent stimulation of insulin and suppression of glucagon release, stimulation of noninsulin-mediated glucose uptake, suppression of endogenous glucose production independent of pancreatic hormones.³⁻⁵

However, due to its peptidic nature and short plasma half-life, GLP-1 must be administered parenterally and continuously to exert its therapeutic action.⁶ Recognition that the enzyme dipeptidyl peptidase-IV (DPP-4) is responsible for the degradation and inactivation of GLP-1 raised the possibility of developing small molecule inhibitors of this enzyme to leverage the antidiabetic effects of endogenous GLP-1 while avoiding the need for parenteral administration.

Several orally available specific inhibitors of DPP-4 have been described and have been reported to improve glucose metabolism in various animal models of type 2 diabetes.⁷⁻⁹ Several antidiabetic drug including dipeptidyl peptidase 4 (DPP-4) inhibitor has shown to exert cardio protective effects in addition to their glycaemic control. A recent study has shown that beneficial effects of DPP-4 Inhibitor on metabolic parameter in type 2 diabetic patients.¹⁰ However, the effects vildagliptin on metabolic syndrome in setting of diabetes model has not been explored.

The present study was designed to evaluate potential effects of vildagliptin on various components of metabolic syndrome (Blood glucose, HbA1c, serum insulin, HOMA- IR, HOMA- β , C-peptide), central obesity (body weight, abdominal circumference (AC), thoracic circumference (TC), AC/TC ratio) and dyslipidemic (lipid profile, artherogenic index) with diabetes as an essential component. Also to understand the underlying mechanisms: DPP-IV pathway (serum DPP-IV), anti-inflammatory (hs-CRP), antioxidant (MDA), cardio protective activities (CPK-MB), safety parameters {pancreatic function [lipase (U/L)], liver function [SGPT (U/L)], renal function [creatinine (mg/dl)]} and histopathological indices of injury in the experimental model of diabetes with metabolic syndrome were studied.

METHODS

Chemicals and drugs

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

Experimental animal

Adult male wistar rats, 10 to 12 weeks old, weighing 150 to 200 gm were used in the study. The rats were housed in the central animal facility of our own MGM Medical College, Navi Mumbai, India. They were maintained under standard laboratory conditions in the animal house. 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. Rats were kept in polyacrylic cages (38×23×15 cm) with not more than four animals per cage and housed in an air-conditioned room, kept under natural light-dark cycles. The animals were allowed free access to standard diet or high fat diet as the case may be and water ad libitum.

Preparation high fat diet

The high fat diet (HFD) was prepared indigenously in our laboratory by using normal pellet diet, raw cholesterol, mixture of vanaspati ghee and coconut oil (2:1). Normal rat pellet diet was powdered by grinding and mixed with 2.5% cholesterol and mixture of vanaspati ghee and coconut oil (5%). The mixture was made into pellet form and put into freezer to solidify. In addition 2% raw cholesterol powder was mixed in coconut oil and administered to the rats by oral route (3 ml/kg).¹¹

Experimental model of diabetes with metabolic syndrome

The high fat diet (HFD) along with 2% liquid cholesterol (3 ml/kg) was orally fed to rats for 3 weeks to induce metabolic syndrome. After 3 weeks of dietary manipulation, overnight fasted rats were injected intraperitoneally with STZ (40 mg /kg).¹² The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. The body weight and biochemical parameters (blood glucose, total cholesterol) were estimated 7 days after the vehicle or STZ injection, i.e., on 4 weeks of dietary manipulation in rats.

The rats with blood glucose (>200 mg/dl), Total Cholesterol (>110 mg/dl), triglyceride (>150 mg/dl), change in body weight (8% of initial weight), and reduced HDL levels (<35 mg/dl) confirmed presence of

metabolic syndrome with diabetes. Thereafter the rats were either fed normal diet or HFD as per the protocol for 10 weeks. Blood samples were collected from the retro-orbital plexus under light anaesthesia at 0, 4, 7 and 10 weeks for estimation of biochemical parameters. At the ends of experimental period, rats were sacrificed for histopathological evaluation of injury to the heart, pancreas, liver and kidney.

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 10 weeks. At the end of 3 week, 0.01 M citrate buffer, pH 4.5 was injected intraperitoneally to mimic the STZ injections.

Group 2: High fat diabetic control (HF-DC)

The HFD was fed to rats for 10 weeks to produce metabolic syndrome. At the end of 3 week diabetes was induced by a single STZ injection (40 mg/kg body weight, i.p. dissolved in 0.01 M citrate buffer, pH 4.5).

Group 2: Vildagliptin (VIL)

The HFD was fed to rats for 10 weeks to produce metabolic syndrome. At the end of 3 week diabetes was induced by a single STZ injection (40 mg/kg body weight, i.p. dissolved in 0.01 M citrate buffer, pH 4.5). The vildagliptin (10 mg/kg) was fed orally to rat from 5th weeks to 10th weeks daily.

Evaluation parameters

Anthropometric parameter

Body weight (gm), abdominal circumference (AC), thoracic circumference (TC), AC/TC ratio was recorded every 4 weeks and the change in these parameters were calculated.

Biochemical Parameters

The rat blood samples of all experimental groups were collected from the retro-orbital plexus under light anesthesia at 0, 4, 7 and 10 weeks for estimation of blood glucose, TC, TG, CPK-MB. In addition, after the completion of the experimental duration (10 weeks), serum was used for the determination of the following parameters like lipid profile, serum insulin, HOMA-IR, HOMA- β , C-peptide, serum DPP-IV, hs-CRP, MDA, pancreatic lipase, SGPT, creatinine by Auto-analyzer or ELISA kits in the Pathology (NABL accredited) and pharmacology laboratory.

Histopathological studies

At the end of the experiment (10 weeks), the animals were sacrificed. The heart, liver, kidney 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 hematoxylin 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.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and values were considered at $P < 0.05$.

RESULTS

Characteristics of the high fat diet and low dose of STZ induced diabetes with metabolic syndrome

The high fat diet increased body weight significantly as compared with the normal control at 3rd weeks. After 3 weeks of dietary manipulation, rats were injected intraperitoneally with STZ (40 mg/kg). The increased Body weight and biochemical parameters (increased Blood glucose, triglyceride, total cholesterol) resulted in obesity, dyslipidaemia and type II diabetes, confirmed the presence of diabetes with metabolic syndrome.

Anthropometric parameter

The HF-DC and vildagliptin group showed significant ($p < 0.001$) increase in body weight at 4th week as compared with NC group rats. The increase in body weight in HF-DC and vildagliptin group rats was not sustained till the end of 10th week. Vildagliptin (10 mg/kg) treated group rats at week 10, showed significant ($p < 0.01$) lower body weight as compared to HF-DC group rats. The weight difference between NC and HF-DC (baseline versus 10th week weight) was found to be 50.91% in NC, 58% in HF-DC and 42.28% vildagliptin. Similarly, the AC and TC of the vildagliptin group rats also reduced significantly ($p < 0.05$) only at 7th and 10th week as compared to the HF-DC. There was no statistical difference between AC/TC ratio in NC, HF-DC and vildagliptin group rats (Table 1).

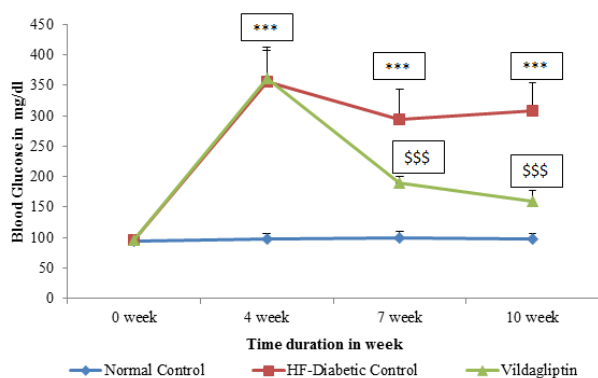
Biochemical parameters

Metabolic parameters

The blood glucose, triglyceride and total cholesterol levels in the HF-DC group rats were markedly higher ($p < 0.001$) as compared to NC group rats. In vildagliptin

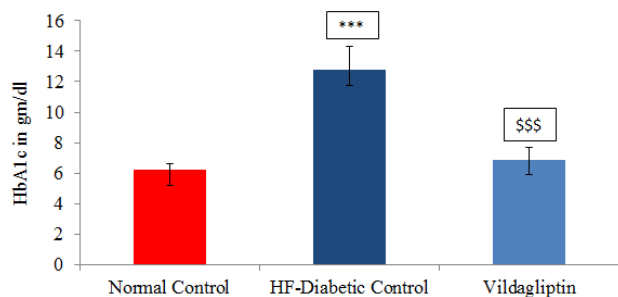
group rats these parameters were significantly lower ($p < 0.001$) as compared to HF-DC group rats at 7th and 10th week. Glycosylated hemoglobin ($p < 0.001$), total cholesterol ($p < 0.001$), triglyceride ($p < 0.001$), low density lipoprotein ($p < 0.001$), HOM-IR, and atherogenic index ($p < 0.01$) were significantly reduced in vildagliptin treated group.

Serum insulin ($p < 0.01$), and HOMA- β ($p < 0.01$) was significantly increased in vildagliptin group as compared with HF-DC group at the end of 10th weeks. Nonetheless, high density lipoprotein was significantly ($p < 0.01$) increased in vildagliptin group rats as compared with HF-DC. The C-peptide levels in vildagliptin group increased though statistically not significant as compared to NC and HF-DC group rats (Figure 1, 2, 3 and Table 2, 3).



Values are expressed as mean \pm SD. *** $p < 0.001$ NC versus HF-DC, \$\$\$ $p < 0.001$ HF-DC versus VIL.

Figure 1: Time course changes of blood glucose level of NC (n=8), HF-DC group (n=7) and VIL group (n=8).



Values are expressed as mean \pm SD. *** $p < 0.001$ NC versus HF-DC, \$\$\$ $p < 0.001$ HF-DC versus VIL.

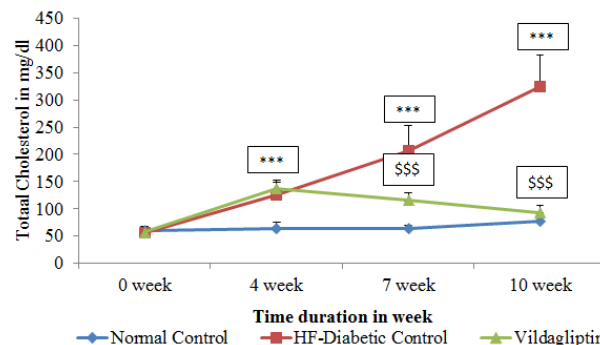
Figure 2: Time course changes of HbA1c of NC (n=8), HF-DC group (n=7) and VIL group (n=8).

Cardiac variables

The CPK-MB levels in different experimental groups did not rise significantly at 4th week. However, there was a significant ($P < 0.001$) reduction in serum CPK-MB levels in vildagliptin group rats at 7th and 10th week as compared with HF-DC (Figure 4).

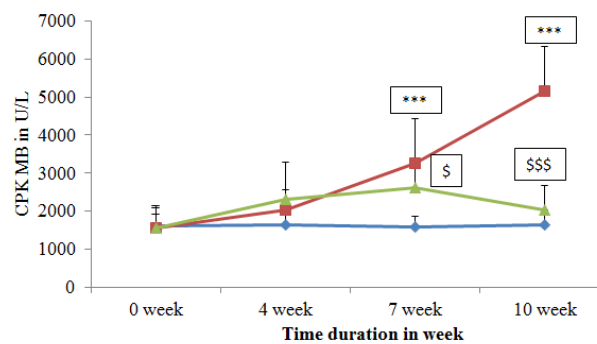
DPP-IV pathway, anti-inflammatory, antioxidant Variables

The serum DPP-IV levels ($p < 0.001$) increased significantly in HF-DC group rats as compared to NC group rats. Vildagliptin treated rats showed significant reduction in serum DPP-IV levels as compared to HF-DC rats. Similarly inflammatory (hs-CRP) ($p < 0.05$), oxidative marker (MDA) ($p < 0.01$) also reduced in vildagliptin group as compared to HF-DC group rats on 10th week (Table 4).



Values are expressed as mean \pm SD. *** $p < 0.001$ NC versus HF-DC, \$\$\$ $p < 0.001$ HF-DC versus VIL.

Figure 3: Time course changes in total cholesterol among experimental groups of NC (n=8), HF-DC group (n=7) and VIL group (n=8).



Values are expressed as mean \pm SD. *** $p < 0.001$ NC versus HF-DC, \$ $p < 0.05$, \$\$\$ $p < 0.001$ HF-DC versus VIL.

Figure 4: Time course changes in CPK-MB of NC (n=8), HF-DC group (n=7) and VIL group (n=8).

Pancreatic, liver and kidney function markers

The vildagliptin group rats did not demonstrate a significant reduction in the level of pancreatic lipase (U/L) as compared with HF-DC, but the treatment group showed reduced level of SGPT (U/L) ($p < 0.01$) and creatinine (mg/dl) ($p < 0.01$) when compared to HF-DC group rats at 10th week (Table 5).

Table 1: Anthropometric parameter among various experimental groups.

Groups	Duration	Variable			
		Body weight	AC	TC	AC/TC
Baseline					
NC		157.63±7.11	14.13±0.49	13.06±00.40	1.081
HF-DC		161.14±5.11	14.28±0.39	13.14±0.55	1.08
Vildagliptin		156.87±9.04	13.93±0.49	12.87±0.44	1.08
4 weeks					
NC		188.87±6.22	15.00±0.26	13.93±0.41	1.076
HF-DC		235.14±4.59 ^{***}	17.72±0.48	16.71±0.48	1.06
Vildagliptin		232.75±9.55	17.18±0.84	16.12±0.91	1.06
7 weeks					
NC		214.12±5.33	16.31±0.25	15.25±0.26	1.069
HF-DC		226.42±4.68 [*]	17.00±0.40	16.00±0.41	1.06
Vildagliptin		218.87±10.86 [*]	16.50±0.59	15.50±0.59	1.06
10 weeks					
NC		237.88±4.99	17.68±0.70	16.62±0.74	1.063
HF-DC		219.14±9.92 [§]	16.58±0.45	15.57±0.47	1.06
Vildagliptin		199.25±8.84	15.43±0.67 [§]	14.37±0.79 [§]	1.07
Weight differences between baseline (T₀) and final (T₁₀th week)					
	Weight baseline (T ₀ week)	Weight final (T ₁₀ th week)	% Difference in weight (T ₁₀ th week- T ₀ week)		
NC	157.63	237.88	50.91		
HF-DC	161.14	219.14	58		
Vildagliptin	156.87	199.25	42.38		

NC: Normal control group (n=8), HF-DC: High fat diabetic control group (n=7) and VIL: Vildagliptin group (n=8). Values are expressed as mean ± SD. *P<0.05, **p<0.01 NC versus HF-DC, §P<0.05, §§p<0.01HF-DC versus VIL.

Table 2: Assessment of insulin resistance parameter in various experimental groups.

Name of parameter	Insulin	C-Peptide	HOMA IR	HOMB-β
NC	6.46±0.65 ^{**}	0.07±0.02	1.57±0.16	66.6±5.86 ^{**}
HF-DC	2.93±1.11	0.05±0.03	2.17±0.63	5.9±2.2
Vildagliptin	4.73±1.45 [§]	0.063±0.03	1.79±0.57	18.37±5.4 ^{§§}

NC: Normal Control group (n=8), HF-DC: High fat diabetic control group (n=7) and VIL: Vildagliptin group (n=8). Values are expressed as mean ± SD. **p<0.01 NC versus HF-DC, §§p<0.01HF-DC versus VIL.

Table 3: Lipid profile in various experimental groups.

Variable	NC	HF-DC	Vildagliptin
TG (mg/dl)	63.75±11.47	312.85±62.24 ^{***}	163.00±108.22 ^{***}
HDL (mg/dl)	32.62±2.56	26.57±5.74 [*]	34.12±5.489 [*]
LDL (mg/dl)	12.6±2.41	62.57±12.44 ^{***}	32.42±21.75 ^{***}
Atherogenic Index	1.36±0.20	11.97±4.76 ^{***}	1.5±0.57 ^{***}

NC: Normal Control group (n=8), HF-DC: High fat diabetic control group (n=7) and VIL: Vildagliptin group (n=8). Values are expressed as mean ± SD. *P<0.05, **p<0.01, ***p<0.001 NC versus HF-DC, §P<0.05, §§p<0.01, \$\$\$p<0.001HF-DC versus VIL.

Table 4: DPP-IV levels, inflammatory and lipid peroxidation marker in various experimental groups.

Name of Parameter	NC	HF-DC	Vildagliptin
Serum DPP-IV (microunit/ml)	4.76±0.48	44.53±5.04 ^{***}	28.45±3.58 ^{§§}
Hs-CRP(mg/dl)	0.86±0.11	2.2±0.52 [*]	0.94±0.16 [§]
MDA(nmol/ml)	1.86±0.08	6.03±0.66 ^{***}	3.54±0.08 ^{§§}

NC: Normal Control group (n=8), HF-DC: High Fat Diabetic Control group (n=7) and VIL: Vildagliptin group (n=8). Values are expressed as mean ± SD. *P<0.05, ***P<0.001 NC versus HF-DC, §P<0.05, §§ P<0.01HF-DC versus VIL.

Table 5: Safety marker in various experimental groups.

Variable	NC	HF-DC	Vildagliptin
Liver marker			
SGPT	62.77±11.58	99.85±10.38***	65.75±8.21***
Kidney marker			
Creatinine	0.35±0.07	1.27±0.43***	0.32±0.05***
Pancreatic marker			
Pancreatic lipase	33.66±4.62	48.26±9.36***	45.5±5.44

NC: Normal Control group (n=8), HF-DC: High fat diabetic control group (n=7) and VIL: Vildagliptin group (n=8). Values are expressed as mean ± SD. *P<0.05, ***p<0.001 NC versus HF-DC, \$P<0.05, \$\$p<0.01 HF-DC versus VIL

Histopathological assessment

Histopathological section of myocardium

Photomicrograph of heart of NC group rat heart revealed the non-infracted architecture of the myocardium [Figure 5 (plate 1A)]. In contrast, HF-diabetic control group rat heart shows fatty infiltration in myocardial cells, hemorrhage, marked edema, confluent areas of myonecrosis separation of myofibers, congested blood vessels and inflammation as compared to the NC group (plate 1B). In the vildagliptin treatment group rats, inflammation, necrosis and edema was observed. However the degree of edema, inflammation and necrosis was less as compared to the HF-diabetic control group (plate 1C) (H and E x 40).

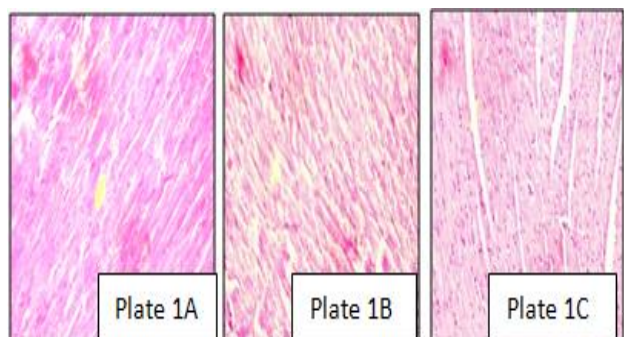


Figure 5: Plate 1(A) to (C) histopathological section of myocardium.

Histopathological section of pancreas

Photomicrograph of pancreas sections of NC rats shows, an organized pattern and shows normal architecture of islets of langerhans and the beta cells [Figure 6 (plate 3A)]. In contrast, the pancreas of HF-diabetic control group rat showed severe degenerative changes in the pancreatic islets, damaged islets of langerhans, reduced beta cell mass and the atrophy of beta cells with the loss of few nucleus and cytoplasm, inflammatory infiltration more was observed (plate 3B). In the vildagliptin treatment group rats pancreas showed,

improved beta cell mass, less inflammatory cells as compared to HF-DC group (plate 3C) (H and E x 40).

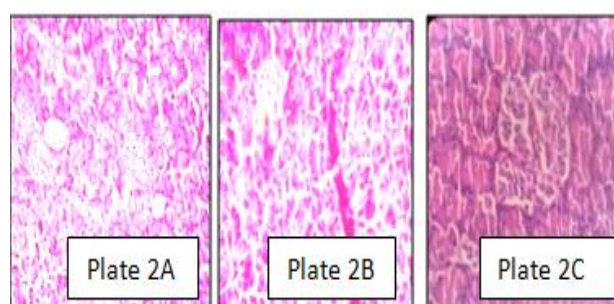


Figure 6: Plate 2(A) to (C) histopathological assessment of the pancreas.

Histopathological section of liver

Photomicrograph of liver sections of NC rats showed, normal architecture of central vein, peripheral vein and no congestion of sinusoides [Figure 7 (plate 3A)]. In contrast, the liver of HF-diabetic control group rat showed fatty liver, moderate fatty degeneration, ballooning of cell, inflammatory infiltration more and congestion of blood vessels in central vein (plate 3B).

In the vildagliptin treatment group rats liver showed, less fatty degeneration, less inflammatory infiltration, congestion of blood vessels, fibrosis, edema and necrosis as compared to HF-DC group (H and E x 40).

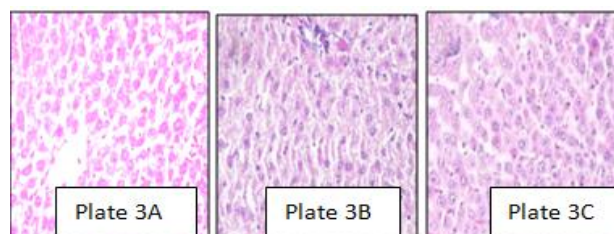


Figure 7: Plate 3(A) to (C) histopathological assessment of the liver.

Histopathological section of kidney

Photomicrograph of renal sections of NC rats shows normal structure of the kidney. There was absence of congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration [Figure 8 (plate 4A)]. In contrast histological assessment of the HF-DC group rat demonstrated congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration as compared to NC group (plate 4B). In vildagliptin treated group kidney shows congestion of glomerular blood vessels, less hemorrhage, less tubular necrosis, inflammation and focal area as compare to HF-DC group (plate 4C) (H and E x 40).



Figure 8: Plate 4(A) to (C) histopathological assessment of kidney.

Immunohistochemistry of pancreas for insulin localisation

Immunohistochemistry of NC group pancreas showed increased localization of Insulin in the NC as compared to HF-DC (plate 5A). The HF-DC group showed loss of beta cell mass resulting in decrease in Insulin secretion (plate 5B). The vildagliptin group showed increase beta cell mass which was functional and secreting insulin (plate 5C).

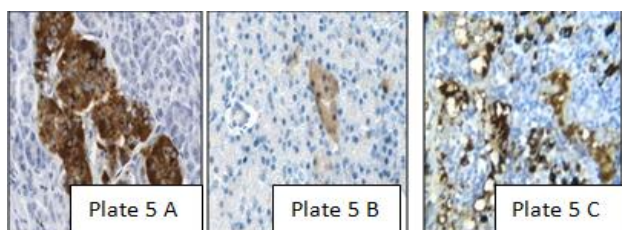


Figure 8: Plate 5(A) to (C) immunohistochemistry of the pancreas.

DISCUSSION

Metabolic syndrome includes central obesity, insulin resistance, elevated blood pressure, impaired glucose tolerance and dyslipidaemia. The number of adults with metabolic syndrome is substantial and the prevalence is increasing throughout the world. In the Indian subcontinent, 45% of males and 38% of females are diagnosed with metabolic syndrome. Majority of individuals diagnosed as metabolic syndrome are also diabetics.

DPP-4 Inhibitors are a novel class of anti-diabetic drugs that are widely being used clinically. DPP-4 Inhibitor like vildagliptin possesses several beneficial effects like reduced blood glucose, inflammation, obesity, cardiac complication and insulin resistance could be beneficial in subset of diabetic patients with metabolic syndrome. Despite these beneficial effects, vildagliptin's therapeutic effect in the setting of diabetes with metabolic syndrome in experimental rats remains unexplored. This is the first report of the efficacy of vildagliptin in experimental model of metabolic syndrome with diabetes.

The significant finding of this study is that vildagliptin ameliorates diabetes with metabolic syndrome induced deleterious changes in experimental rats. Vildagliptin treatment favorably modulated diabetes (blood glucose, restoration of pancreatic function), central obesity (body weight, abdominal circumference (AC), thoracic circumference (TC), AC/TC ratio) and hypolipidemic (favorable lipid profile, artherogenic index), cardioprotective (CPK-MB) parameters in the experimental model of diabetes with metabolic syndrome. Also to understand the mechanisms; DPP-IV pathway (serum DPP-IV), 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 metabolic syndrome was studied.

Essential components of Metabolic Syndrome with diabetes

Diabetes

In our study the rats fed with HFD/STZ showed significant increase in blood glucose glycosylated hemoglobin, reduced serum insulin levels, which was ameliorated after administration of Vildagliptin at the end of 10 weeks. The previous study by Burkey et al.¹² showed similar results by significantly reversing the HFD/STZ induced increase in blood glucose levels. The vildagliptin treatment showed that HOMA-IR was reduced whereas HOMA- β was increased. Study by Kuate D et al supported our finding.¹³ The C-peptide determined by Nora M. El- Sheikh showed reduced level of C-peptide in diabetic rats similar to present study which is restored by treatment with vildagliptin.¹⁴ Type 2 diabetes is characterized by progressive β cell destruction, as a result of chronic insulin resistance and the loss of β cell mass and function, as demonstrated by previous studies.¹⁵ The primary mechanism underlying the reduction in β cell mass in patients with T2DM is the apoptosis of β cells in all diabetic patients.¹⁶ The present study observed that the vildagliptin treatment showed restoration of β cell mass as compared to HF-DC group.

Central obesity

Various anthropometric parameters such as body weight, thoracic circumferences (TC), abdominal circumference (AC), and their ratios (AC/TC) were evaluated in the normal control (NC), high fat diabetic control (HF-DC) and vildagliptin groups. The weight difference between NC, HF-DC and vildagliptin on baseline weight and 10th week weight are 50.91% in NC, 58% in HF-DC and 42.28% vildagliptin. Similarly, the anthropometric results may be attributed to the diabetic state that is known to cause weight loss as shown by Kuate D et al.¹³

Dyslipidemia

The role of dyslipidemia in the development of diabetes macrovascular complications is well known. In our study, the HFD/STZ-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 Nicolas F. Renna et al showed treatment with DPP-4 Inhibitor significantly reduced the TC, TG, LDL-C level and increased HDL-C levels in HFD/STZ rats.¹⁷ The vildagliptin treatment also showed favorable effects on atherogenic index.

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 high fat diet and STZ in rats. However, treatment with vildagliptin significantly restored increase in serum CPK-MB levels at 7th and 10th week. The study by Shamim A et al showed increase in the cardiac marker enzymes CPK-MB in diabetic high fat diet rat.¹⁸ The myocardial injury induced by high fat diet and STZ shown by biochemical marker was also confirmed by histopathological assessment.

Mechanism; DPP-IV pathway, inflammatory, oxidant Variables

Due to the recognized benefits of prolonging the biological actions of GLP-1, DPP-IV inhibition has been recognized as a possible mechanistic approach to the treatment of type II diabetes. In our study HFD/STZ treated with vildagliptin rats showed reduced serum DPP-IV level in setting of diabetes with metabolic syndrome. Burkey BF et al showed reduced level of DPP-4 activity in insulin resistant Rats, which states similar finding with present study.¹²

Similar to the present findings Renna NF also found raised hs-CRP in Fructose fed hypertensive rats as compared with normal rats. Similar results were shown by present study.¹⁷ The end product of oxidative stress revealed that obesity and type II diabetes enhanced lipid peroxidation. In the present study, MDA was increased

significantly in HFD/STZ group rats. The vildagliptin treatment significantly modulated these parameters and showed its antioxidant activity. Our data are in accordance with the previous report by Renna NF et al and Kuate et al.^{13,17}

Safety variable

Pancreatic Lipase was assessed to detect pancreatic damage. Increased Lipase levels as seen in HFD/STZ rats showed presence of pancreatic tissue damage as compared to NC. The pancreatic lipase is not significantly reduced in Vildagliptin treatment group as compared with HF-DC but histopathology of pancreas showed restoration in the architecture of the pancreas.

In the present study HFD/STZ treated rats showed increased levels of SGPT liver enzyme. Numerous studies have reported that diabetes is associated with raised levels of SGPT. In a large clinical study reported by Erbey JR et al patients who were overweight (BMI 25-30 kg/m²) and obese (BMI >30 kg/m²) were more likely to have elevated SGPT levels.¹⁸ There was 10.6% prevalence in obese diabetic patients versus 6.6% prevalence in obese non diabetic patients. As per, biochemical and histopathological report, the vildagliptin group rats showed no adverse effects on liver function.

In addition, recent evidence suggests that diabetic is associated with changes in morphology and eventually functional alteration in kidneys. The present results clearly demonstrate raised kidney function marker creatinine in serum of HFD/STZ group. In contrast, the HFD/STZ Vildagliptin treated rats showed significant reduction in these markers, thus showing its ability to protect against high fat diet diabetes-induced renal damage. The present study also confirmed the protective effects on pancreas, liver and renal function as shown by biochemical findings and histopathological assessment of pancreases, liver and kidney.

Thus, the beneficial effects of vildagliptin (10 mg/kg) as shown by present study reveals its protective effects on deleterious changes induced by diabetes with metabolic syndrome via multiple mechanisms: hypoglycemic, hypolipidemic, antioxidant, cardioprotective, anti-inflammatory and DPP-IV Inhibitory property.

CONCLUSION

Vildagliptin treatment reveals, the hypoglycemic, hypolipidemic, antioxidant, cardioprotective, anti-inflammatory and DPP-IV Inhibitory properties in the experimental model which may contribute to its beneficial effects in the setting of diabetes with metabolic syndrome. Restoration of beta cell mass and function may additionally attribute to the usefulness of Vildagliptin in the experimental model.

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