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Research Article

Cardiometabolic Efficacy and Mechanisms of Berberine in Experimentally Induced Changes in Diabetes and Metabolic Syndrome Rats

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Abstract

Background: Berberine is natural plant alkaloid and is known to possess independent anti-diabetic and hypolipidemic activities but its efficacy is unknown in setting of diabetes co-existing with metabolic syndrome.

Objective: The present study was designed to evaluate the potential beneficial effects of Berberine in an experimental model of diabetes co-existing with metabolic syndrome

Methods: A combination of High Fat Diet (HFD) and low dose of streptozotocin (STZ) 40 mg/kg was used to induce metabolic syndrome and diabetes mellitus in Wistar rats. After the confirmation of metabolic syndrome in the setting of diabetes, Berberine 100 mg/kg was orally fed to rats from 4th to 10th week. After the completion of the study duration various biochemical and histopathological parameters were estimated.

Results: Berberine treatment does not have any significant effect on body weight as compared with HF-DC group. Nonetheless, Berberine treatment showed beneficial effects on metabolic parameter like diabetes (significant reduction in blood glucose, HbA1c, increase in serum insulin and C-peptide, reduction in HOMA-IR, and increase in HOMA-β), dyslipidemia (significant decrease in TG, TC, LDL-C, and increase in HDL-C), cardioprotection (reduction in CPK-MB, hs-CRP, Atherogenic index) as compared with HF-DC group. Berberine treatment also significantly reduced serum DPP-IV and MDA levels which could contribute to its beneficial in diabetes with metabolic disorder. The decrease in serum DPP-IV level correlated to an increase in insulin levels.

Berberine treatment did not adversely affect hepatic (SGPT (U/L) levels), renal (creatinine levels) and pancreatic function (pancreatic lipase levels). The Immunohistochemical localization of the pancreas suggested that Berberine preserved pancreatic beta cell mass.

Conclusion: The present study has demonstrated antidiabetic, hypolipidemic effects, cardioprotective, antioxidant property of berberine in the setting of diabetes with metabolic syndrome. These beneficial effects may be attributed to the DPP-IV inhibitory activity of Berberine.

Keywords: *Berberine; Diabetes; Metabolic Syndrome; High Fat Diet; Streptozotocin; Rats*

Introduction

The metabolic syndrome (MetS) is a major and escalating public-health and clinical challenge worldwide in the wake of urbanization, surplus energy intake, increasing obesity, and sedentary life habits. The International diabetes federation (IDF) estimates that one-quarter of the world's adult population has the MetS [1]. Metabolic syndrome confers a 5-fold increase in the risk of type II diabetes mellitus and 2-fold the risk of developing cardiovascular disease over the next 5 to 10 years [2], thus their etiologies, preventions and therapies

have become a matter of global research interest. The concept of metabolic syndrome was generated by Kylin in 1923, who described a cluster of medical conditions, such as hypertension, hyperglycemia and gout [3]. The concept did not attract much attention until Dr. Gerald Reaven introduced the syndrome X in 1988 [4], which is similar to the metabolic syndrome. In 1999, WHO (World Health Organization) and EGIR (European Group for the Study of Insulin Resistance) released their diagnostic criteria for the metabolic syndrome, respectively [5,6]. Now, there are at least 6 sets of diagnostic criteria for the syndrome from different organizations over the world. The primary contents of diagnostic criteria are similar among these organizations. They are hyperglycemia, insulin resistance, central obesity, hypertension, elevated triglycerides and decreased high-density lipoprotein-cholesterol.

The use of natural products for the treatment of metabolic syndrome has not been explored in depth despite the fact that a number of modern medication available in market. Berberine, an isoquinoline alkaloids originally isolated from the root of *Berberis aristata* belongs to family berberidaceae and has shown to display a wide array of pharmacological activities including antimicrobial, antitumor, anti-inflammation and Antidiabetic [7-10]. In 1988, the hypoglycemic effect of Berberine was found when Berberine was used to treat diarrhea in diabetic patients in China [11]. Since then, Berberine has been used as an anti-hyperglycemic agent. In addition to its beneficial effects in diabetes, in one recent single-blind clinical observation, the study showed that diet supplementation of natural substance Berberine was beneficial for correcting lipid metabolism disorders and reducing cardiovascular risk factors [12]. In the present study, we have focused on Berberine because this natural product has been reported in the several literature to have independent beneficial effects in human type II diabetes as well as lipid metabolism. Strikingly, Berberine acutely stimulated AMPK activity in both myotubes and adipocytes *in vitro*, contributing to enhanced GLUT4 translocation in myotubes and reduced lipid mass in adipocytes [13,14]. Based on these studies, we propose that Berberine may have a major application as a new treatment for diabetes co-existing with metabolic syndrome.

Dipeptidyl peptidase-IV (DPP-IV) degrades glucagon-like peptide-1, which plays a critical role in insulin secretion and signaling [15]. In general, obese subjects present much higher risk of developing insulin resistance and diabetes. Coincidently, a twofold increase in DPP-IV release is observed in obese subjects [16]. Interestingly, plasma DPP-IV activity increases in rats after STZ injection and positively correlates with blood glucose levels [17]. Berberine was investigated as an inhibitor of human DPP-IV in an attempt to explain its anti-hyperglycemic activities. The investigation included simulated docking experiments to fit berberine within the binding pocket of DPP-IV. Experimentally, berberine was found to inhibit human recombinant DPP-IV *in vitro* with IC (50) = 13.3 microM. The findings suggest that DPP-IV inhibition is, at least, one of the mechanisms that explain the anti-hyperglycemic activity of Berberine. The fact that Berberine was recently reported to potently inhibit the pro-diabetic target human protein tyrosine phosphatase 1B (h-PTP 1B) discloses a novel dual natural h-PTP 1B/DPP-IV inhibitor [18]. The cardioprotective effects of berberine perhaps due to its DPPIV Inhibitory activity in the experimental models of I-R injury have been previously reported [19-21].

However, in spite of the reported DPPIV Inhibitory activity of berberine no studies have been carried out to investigate if this *in vitro* DPPIV Inhibitory activity of Berberine translates into beneficial therapeutic effects in the setting of diabetes co-existing with metabolic syndrome. In the light of the above, the present study was undertaken to investigate the effects of Berberine on various components of metabolic syndrome with diabetes as an essential co-morbidity viz. anti-diabetic (blood glucose, HbA1c, serum insulin, HOMA IR, HOMA β, C-peptide), central obesity (percentage change in body weight, AC/TC ratio) and hypolipidemic (lipid profile, atherogenic index). In addition to understand the underlying mechanisms; DPP-IV pathway (serum DPP-IV), anti-inflammatory (hs-CRP), antioxidant (MDA), cardioprotection (CPK-MB) and beta cell preservation (Insulin immunohistochemistry) contributing to the beneficial effects in diabetes with metabolic syndrome was also studied. Also the safety parameter {pancreas [lipase (U/L)], liver [SGPT (U/L)], renal [creatinine (mg/dl)} function and histopathological indices of injury were evaluated to better understand the mechanisms by which Berberine mitigates deleterious changes in diabetes coexisting with metabolic syndrome.

Material and Methods

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 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.*

Chemicals and drugs: Streptozotocin (STZ) and Berberine were procured from Sigma Chemicals St Louis, USA. Cholesterol was procured from Alfa Aesar, Germany. The standard test drugs Metformin and Vildagliptin were obtained as gift samples. All other chemicals and reagents used were of analytical grade.

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) [22].

Experimental model of diabetes with metabolic syndrome: The 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). 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 and triglyceride) 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) 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 anesthesia at 0, 4, 7 and 10 weeks for estimation of biochemical parameters. At the ends of experimental period, rats were sacrificed for histopathological and immunohistochemical evaluation of injury to the heart, aorta, pancreas, liver and kidney [22].

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, 0.01M 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 weeks, diabetes was induced by a single STZ injection (40 mg/kg body wt, i.p. dissolved in 0.01M citrate buffer, pH 4.5).

Group 3: Metformin (MET): 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 wt, i.p. dissolved in 0.01M citrate buffer, pH 4.5). Metformin (100 mg/kg) was fed orally to rat from 5^{th} weeks to 10^{th} weeks daily.

Group 4: 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 wt, i.p. dissolved in 0.01M citrate buffer, pH 4.5). The Vildagliptin (10 mg/kg) was fed orally to rat from 5^{th} weeks to 10^{th} weeks daily.

Group 5: Berberine (BER): 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 wt, i.p. dissolved in 0.01M citrate buffer, pH 4.5). Berberine (100 mg/kg) was fed orally to rat from 5^{th} weeks to 10^{th} weeks daily.

Evaluation parameters

DPP-IV assay reaction

DPP-IV assay was performed using DPP-IV assay kit procured from Sigma-Aldrich. In this assay, DPP-IV activity was determined by the cleavage rate of 7-amino-4-methylcoumarin (AMC) from the synthetic substrate H-glycyl-prolyl-AMC. One unit of DPP-IV is the amount of enzyme that hydrolyzes the DPP-IV substrate to yield 1.0 U mole of AMC per minute at 37°C. The standard curve of free AMC was generated using 0 - 50 mM AMC (Sigma). DPP-IV activity was expressed as the amount of cleaved AMC per minute per ml (nmol/min/ml). The 1% (w/v) Berberine in distilled water was used for the assay. While Sitagliptin and Vildagliptin were used as a reference drugs and control was prepared without inhibitors/test agent. Experiments were done in triplicates. A decrease in DPP-IV activity is measured for inhibition. The percent inhibition was calculated using following formula:

$$\% \text{ Inhibition} = \frac{\text{Control- Inhibitor}}{\text{Control}} \times 100$$

Anthropometric parameter: Percentage change in Body weight and AC/TC ratio were recorded every 4 weeks. Percentage change in body weight expressed according to the following:

% change in body weight = Final weight –Initial weight X 100/Initial weight

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, hs-CRP, MDA, Serum DPP-IV, SGPT, creatinine by Auto-analyzer or ELISA kits in the Pathology (NABL accredited) and Pharmacology laboratory. Both insulin resistance and β-cell function were calculated by homeostasis model assessment (HOMA) [23]: Insulin resistance (HOMA-IR) = (serum glucose, mmol/L x serum insulin, μIU/ml)/22.5 and β- Cell function = (serum insulin, μIU/ml x 20)/(serum glucose, mmol/L) - 3.5. and atherogenic index was calculated from the following formula Atherogenic index (AI) = TC-HDL-C/HDL-C.

Histopathological studies: At the end of the experiment (10 weeks), the animals were sacrificed. The heart, aorta, pancreas, liver and kidney 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 mm 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.

Immunohistochemical localization of insulin: The pancreas was immediately fixed in 10% buffered neutral formalin solution after scarification (10 weeks). The tissues were carefully cut, 3-micrometer thick, and obtained on poly-L-lysine coated slides and transferred to three changes of xylene for 30 minutes, followed by rehydrating with decreasing grades of alcohol. The Antigen Retrieval was in microwave oven, 800 watt for 10 minutes, 420 watt for 10 minutes, and 360 watt for 5 minutes in Citrate buffer pH 6. Immuno-staining was done by Peroxidase block with 3% hydrogen peroxide in methanol for 5 minutes and incubated sections for 10 minutes. Primary Antibody Incubation was undertaken for 30 minutes at room temperature and thereafter incubated with super enhancer for 10 minutes. The tissues were incubated with poly-HRP for 30 min followed by substrate DAB. The slides were then visualized under light microscope to study the immunohistochemical localization of insulin.

Statistical Analysis: The Data were analyzed by One –way analysis of variance (ANOVA) and values less than $p < 0.05$ were considered as statistically significant.

Results

DPP-IV *in vitro* assay of Berberine

The Berberine and synthetic DPP-IV inhibitors (Sitagliptin and Vildagliptin) were screened for DPP-IV inhibitory activity *in vitro* assay using ELISA kit. DPP-IV inhibitory activity of synthetic drugs Vildagliptin and Sitagliptin were found to be 90.42 ± 7.84% and 84.67 ± 8.21%, respectively. The DPP-IV inhibitory activity of Berberine was found to 85.95 ± 7.81%, which is comparable to DPP-IV inhibitory activity of reference standards Vildagliptin and Sitagliptin (Figure 1).

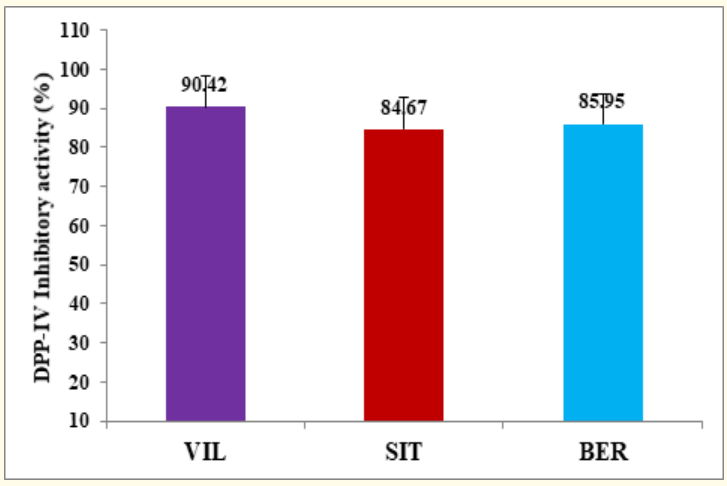

Figure 1: *DPP-IV Inhibitory activity of Berberine and Standard Synthetic DPP-IV Inhibitor (Vildagliptin and Sitagliptin)*

Effect of Berberine on Anthropometric parameters

High Fat Diet with Streptozotocin induced type II diabetes co-existing with metabolic syndrome was associated with altered anthropometric and biochemical parameters (increased blood glucose, triglyceride, total cholesterol) resulting in obesity, dyslipidaemia and type II diabetes.

Administration of Berberine and standard drugs Metformin and Vildagliptin successfully reversed all the param-eters altered by consumption of the HFD/STZ. The change in body weight in NC was found to be 50.91%, HF-DC 40.15%, MET 20.63%, VIL 29.03% and BER 41.49.%. However, chronic treatment with Berberine for 10 weeks significantly ($p < 0.05$) restored the body weight loss as compared to the MET and VIL. However there was no statistical difference between AC/TC ratio of different experimental group rats (Figure 2, Table 1).

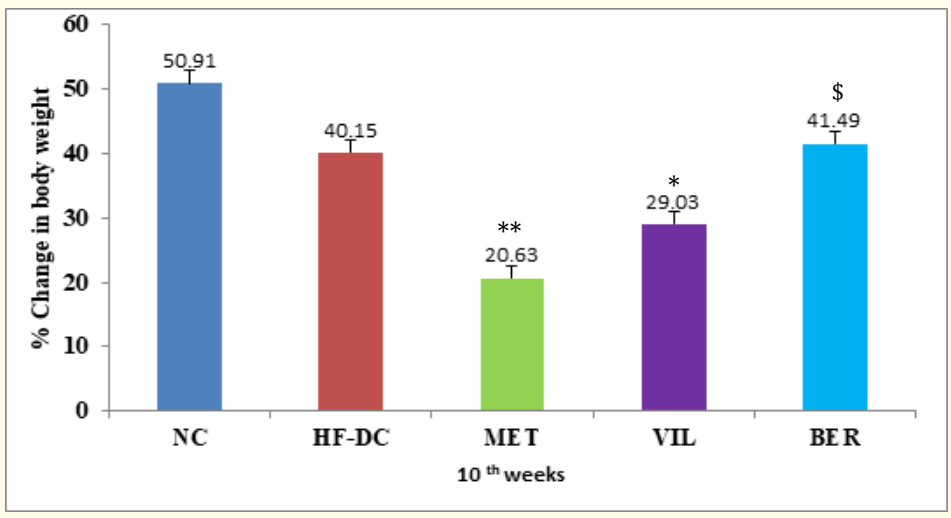

Figure 2: *% change in body weight NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. *p < 0.05; **p < 0.01; VS HF-DC;$ p < 0.05 Vs MET.*

SN	Variable	NC	HF-DC	MET	VIL	BER
1	AC/TC Ratio	1.06	1.06	1.06	1.07	1.06
2	TC (mg/dl)	64.75 ± 12.02	316.57 ± 34.5	105.14 ± 13.01***	98 ± 8.64***	94 ± 14.13**$
3	TG (mg/dl)	74.87 ± 17.37	364.57 ± 33.4	141.57 ± 18.6***	130.12 ± 16.9***	128.85 ± 25.41**$
4	HDL (mg/dl)	32.62 ± 2.55	25.57 ± 2.32	35.71 ± 2.97**	38.12 ± 3.9**	35.71 ± 5.4***$@
5	LDL (mg/dl)	12.6 ± 1.41	62.57 ± 15.68	34.82 ± 3.16**	32.42 ± 2.94**	27.6 ± 7.15**$
6	Atherogenic Index	0.97 ± 0.25	12.16 ± 2.52	2.25 ± 0.49**	1.62 ± 0.51*	1.65 ± 0.33*$

Table 1: *Anthropometric and Lipid profile parameter in various experimental Groups.*

*NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. ***p < 0.001; **p < 0.01; VS HF-DC, $p < 0.05; Vs MET. @p < 0.05 VS VIL*

Effect of Berberine on Biochemical Parameters

Metabolic Parameters

HFD/STZ treatment resulted in significant (p < 0.001) elevation of blood glucose, triglyceride, total cholesterol levels, LDL–C and reduction in HDL-C levels as compared to the NC rats as noted at different periods of the study. As shown in figure 3, MET treatment significantly decreased the high glucose levels followed by VIL and BER treatment as compared to HF-DC. Similar results were also observed in the glycosylated hemoglobin levels (Figure 4).

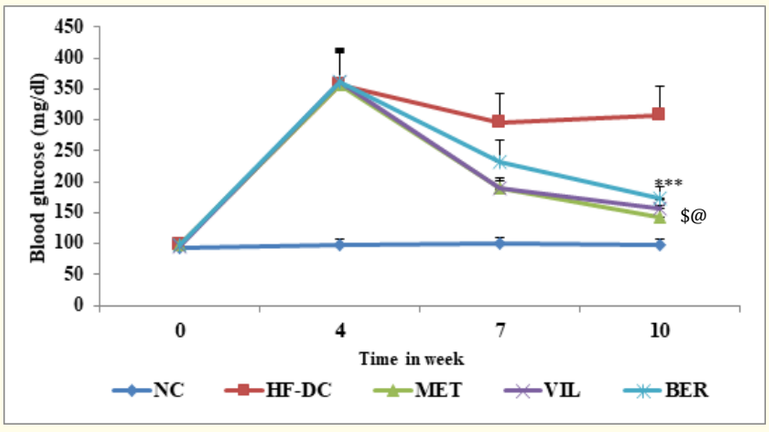

Figure 3: *Time course changes of Blood glucose of NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. ***p<0.01; VS HF-DC; $ p<0.05 Vs MET @p < 0.05 VIL.*

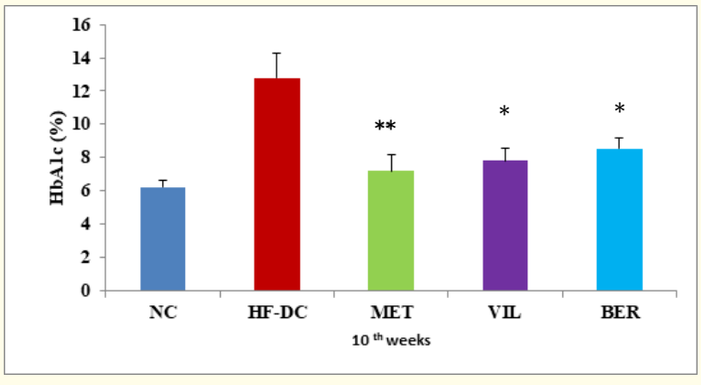

Figure 4: *The HbA1c level at 10th week of NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. **p < 0.01; *P < 0.05 VS HF-DC.*

Serum insulin, was significantly (p < 0.001) increased in HF-DC group as compared with NC group at the end of 10 weeks. HOMA-IR increased in HF-DC whereas HOMA-β reduced significantly in HF-DC group as compared with NC. MET,VIL and BER reduced the HOMA-IR scores in HFD/STZ treated rats. Also the treatment groups restored beta function as indicated by increase in the HOMA- β as compared with HF-DC at 10 weeks. The C-peptide levels in HF-DC were decreased though statistically not significant as compared to NC and other groups (Table 2).

SN	Name of Parameter	Insulin (μU/ml)	C-Peptide (ng/ml)	HOMA IR	HOMB-β	Serum DPP-IV (microunit/ml)	hs-CRP (mg/dl)	MDA (nmol/ml)
1	NC	6.46 ± 0.65	0.07 ± 0.02	1.57	66.6	4.76 ± 0.43	0.86 ± 0.07	1.84 ± 0.08
2	HF-DC	2.93 ± 1.11	0.05 ± 0.03	2.17	5.9	44.53 ± 4.5	2.2 ± 0.5	6.07 ± 0.66
3	MET	4.33 ± 1.2**	0.07 ± 0.02	1.64	17.2*	28.45 ± 2.9**	1.68 ± 0.31*	4.25 ± 0.43*
4	VIL	4.73 ± 0.31**	0.06 ± 0.03	1.79	18.37*	12.32 ± 1.02*$	0.94 ± 0.16*	4.09 ± 0.46*
5	BER	4.06 ± 0.5**	0.06 ± 0.01	1.74	13.26*	18.4 ± 1.36*$	1.15 ± 0.21*	3.54 ± 0.08**

Table 2: *Assessment of Insulin, C-peptide, DPP-IV pathway, inflammatory and oxidant variables in various experimental Groups. NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n=8) and BER (n = 7). Values are expressed as mean ± SD. **p < 0.01; VS HF-DC; $p < 0.05 Vs MET.*

The total cholesterol, triglyceride, low-density lipoprotein and atherogenic index were significantly ($p < 0.001$) increased in HF-DC group as compared with NC group at the end of 10 weeks. HDL was significantly decreased in HF-DC as compared with NC. Total cholesterol ($p < 0,01$), triglyceride ($p < 0.01$), LDL ($p < 0.01$), and atherogenic index ($p < 0.01$) were significantly reduced in BER and Standard drugs treated group as compared with HF-DC group at the end of 10 weeks. HDL was significantly ($p < 0.01$) increased in treated group rats as compared with HF-DC. BER treated rats favorably ($p < 0.05$) modulated lipid parameters as compared to MET treated rats (Table 1).

Cardiac Variables

The results of the study showed a marked increase ($P < 0.001$) in plasma CK-MB isoenzyme level in HF-DC rats when compared to NC group at 7 and 10 weeks. MET, VIL and BER treated group significantly ($P < 0.01$) reversed the HFD/STZ induced increase in CPK-MB levels. A marked protection against cardiac damage was observed as indicated by decrease in this CK-MB isoenzyme level in VIL treated rats as compared to HF-DC administered rats (Figure 5).

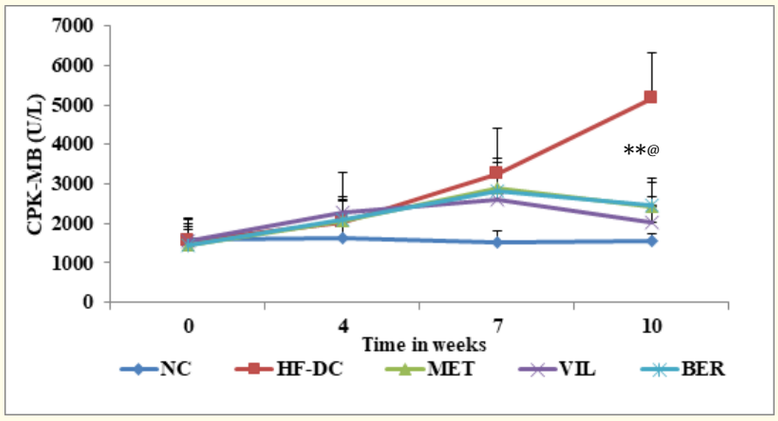

Figure 5: *Time Course Changes in CPK-MB NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. ***p < 0.001 VS HF-DC, @p < 0.05 VS VIL.*

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. MET, VIL and BER treated rats showed significant reduction in serum DPP-IV levels as compared to HF-DC rats. VIL and BER treated rats showed superior reduction in serum DPP-IV level as compared to MET treated rats. Similarly inflammatory (hs-CRP) ($p < 0.01$), and oxidative stress (MDA) ($p < 0.01$) markers were also significantly reduced in treatment group as compared to HF-DC group rats at 10^{th} week (Table 2).

Liver, kidney and pancreatic function markers

As seen from the table 3, it was found that the HFD/STZ treated groups markedly elevated the levels of pancreatic lipase (U/L) ($p < 0.001$), SGPT (U/L) ($p < 0.001$) and creatinine (mg/dl) ($p < 0.001$) at 10th week compared to NC and other treated groups. However clinically increase in pancreatic lipase, SGPT and creatinine are not significant. The treatment groups did not adversely affect the pancreatic, liver and kidney function (Table 3).

SN	Variable	NC	HF-DC	MET	VIL	BER
1	Pancreatic Marker Pancreatic Lipase (U/L)	30.66 ± 2.10	48.26 ± 9.36	36.83 ± 1.50*	44.06 ± 5.44**	34.23 ± 2.05*@
2	Liver Marker SGPT (U/L)	61.25 ± 8.68	99.85 ± 10.38	77.42 ± 7.20**	75.25 ± 6.84**	69.76 ± 6.37*@
3	Kidney Marker Creatinine (mg/dl)	0.32 ± 0.07	1.27 ± 0.43	0.51 ± 0.06*	0.58 ± 0.04*	0.38 ± 0.07*$@

Table 3: *Shows Safety marker in various experimental groups.*

*NC (n = 8), HF-DC (n = 7), MET (n = 8) VIL (n = 8) and BER (n = 7). Values are expressed as mean ± SD. ***p < 0.0) 1 VS HF-DC $ p < 0.05 VS MET @p < 0.05 VS VIL.*

Histopathological assessment

Plate 1 A-E: Histopathological section of myocardium

Photomicrograph of heart of NC group rat heart revealed the non-infracted architecture of the myocardium (Plate 1A). In contrast, HF-DC 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 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 HF-DC (Plate 1C,1D and 1E) (H&E x 40).

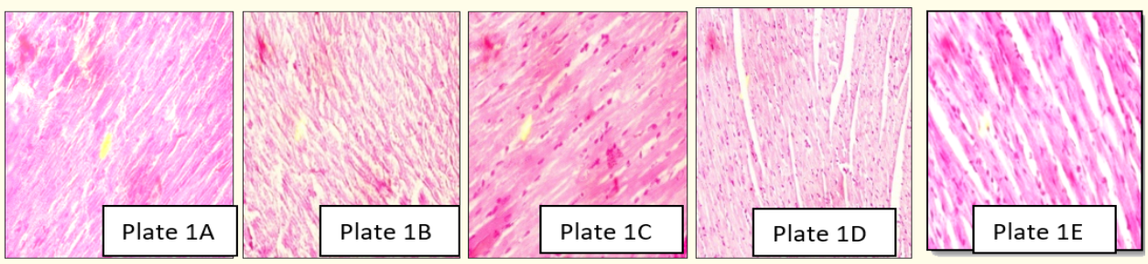

Plate 1: *1A: Photomicrograph of heart of NC group rat heart revealed the non-infracted architecture of myocardium. 1B:HF-DC group rat heart shows fatty infiltration in myocardial cells, hemorrhage, marked edema, congested blood vessels and inflammation. 1C: MET group rats heart shows, occasional focal myofiber loss and the degree of edema, inflammation and necrosis was less as compared to the HF-DC. 1D: VIL group rats heart shows, the degree of edema, inflammation was found but less 1E: BER group rats heart shows, edema, inflammation and necrosis was less as compared to the MET group.*

Plate 2 A-E: Histopathological section of Aorta

Photomicrograph of aorta sections of NC group rats shows no histological changes that is, normal alignment of tunica media, tunica intima, and tunica adventitia as seen (Plate 2A). In case of HF-DC group rats aorta shows, focal and vacuolated cells, inflammation, edema, necrosis were seen in aortic layer and atherosclerotic deposition in the vessel wall (Plate 2B). In the MET treatment group rats aorta shows, less fatty tissues, inflammation, edema hemorrhage were observed as compared to HF-DC (Plate 2C). In the VIL treatment group rats aorta shows, normal alignment of tunica media, tunica intima, and tunica adventitia with less hemorrhage and reduced fat cell as compared to Metformin treated groups (Plate 2D). In the BER treatment group rats aorta shows well maintain alignment of aortic layer with intact intima (Plate 2E) (H&E x 40).

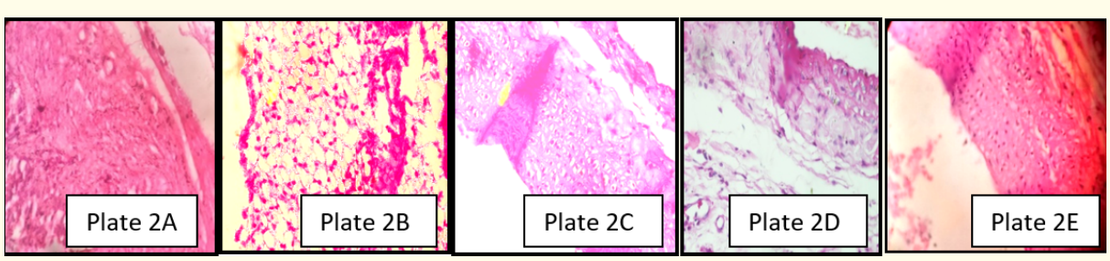

Plate 2: *2A: Photomicrograph of aorta sections of NC group rats shows no histological changes aortic layer. 2B: HF-DC group rats aorta shows, focal and vacuolated cells and atherosclerotic deposition in the vessel wall. 2C: MET group rats aorta shows, less fatty tissues, inflammation, edema hemorrhage were observed as compared to HF-DC. 2D: VIL group rats aorta shows, normal alignment of tunica media, tunica intima, and tunica adventitia with less hemorrhage and reduced fat cell as compared to Metformin treated groups 2E: BER group rats aorta shows well maintain alignment of aortic layer with intact intima.*

Plate 3 A-E: 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 (Plate 3A). In contrast, the pancreas of HF-DC group rat shows severe degenerative changes in the pancreatic islets, damaged islets of langerhans, reduced beta cell mass and the atrophy of beta cells and inflammatory infiltration more was observed (Plate 3B). In the treatment group rats pancreas showed improved beta cell mass, less fibrosis, less inflammatory infiltration and hemorrhage as compared to HF-DC group (Plate 3C, 3D and 3E) (H&E x 40).

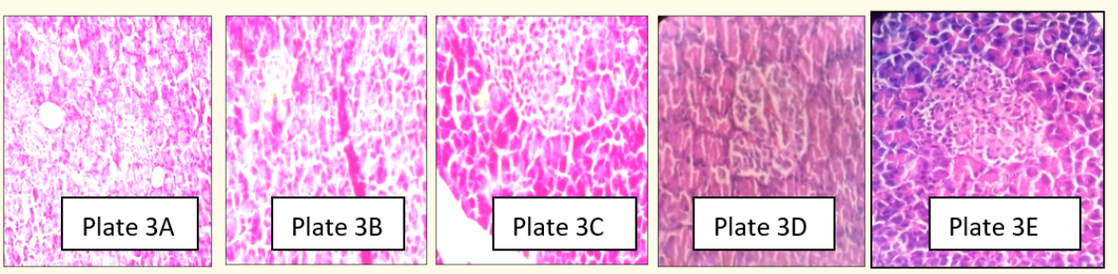

Plate 3: *3A: Photomicrograph of Pancreas sections of NC rats shows, an organized pattern and shows normal architecture Pancreas. 3B: Pancreas of HF-DC group rat shows severe degenerative changes in the pancreatic islets, damaged islets of langerhans, reduced beta cell mass and the atrophy of beta cells 3C: MET group rats pancreas shows, less inflammatory infiltration and hemorrhage as compared to HF-DC group. 3D: VIL group rats pancreas shows, fibrosis, less inflammatory infiltration and hemorrhage 3E: BER group rats pancreas shows, improve beta cell mass less fibrosis, less inflammatory infiltration and no hemorrhage.*

Plate 4 A-E: Histopathological section of Liver

Photomicrograph of liver sections of NC rats showed normal architecture of central vein, peripheral vein and no congestion of sinosoides (Plate 4A). In contrast, the liver of HF-DC group rat showed fatty liver, moderate fatty degeneration, ballooning of cell, inflammatory infiltration and congestion of blood vessels in central vein (Plate 4B). In the treatment group rats liver showedless fatty degeneration, inflammatory infiltration, congestion of blood vessels, fibrosis, edema and necrosis as compared to HF-DC group (Plate 4C, 4D and 4E) (H&E x 40).

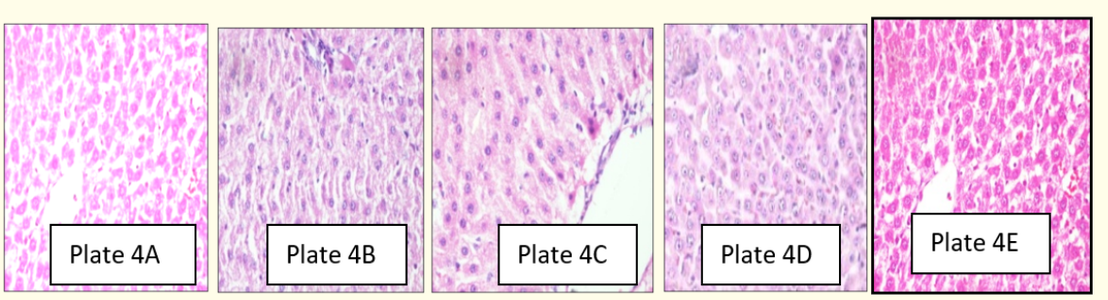

Plate 4: *4A: Photomicrograph of Liver sections of NC rats shows, normal architecture live.4B: the liver of HF-DC group rat shows Fatty liver, moderate fatty degeneration, ballooning of cell, inflammatory infiltration more and congestion of blood vessels in central vein.4C: MET group rats liver shows, less fatty degeneration, inflammatory infiltration, congestion of blood vessels, fibrosis, edema and necrosis. 4D: VIL group rats liver shows, less fatty degeneration, inflammatory infiltration, edema and necrosis. 3E: BER group rats liver shows, less inflammatory infiltration, edema and normal structure of central vein, peripheral vein and no congestion of sinosoides.*

Plate 5A-E: Histopathological section of Kidney

Photomicrograph of kidney sections of NC rats showed normal structure of the kidney. There was absence of congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration (Plate 5A). 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 5B). In treatment group, kidney showed less congestion of glomerular blood vessels, less hemorrhage, less tubular necrosis and inflammation as compared to HF-DC group (Plate 5C, 5D and 5E) (H&E x 40).

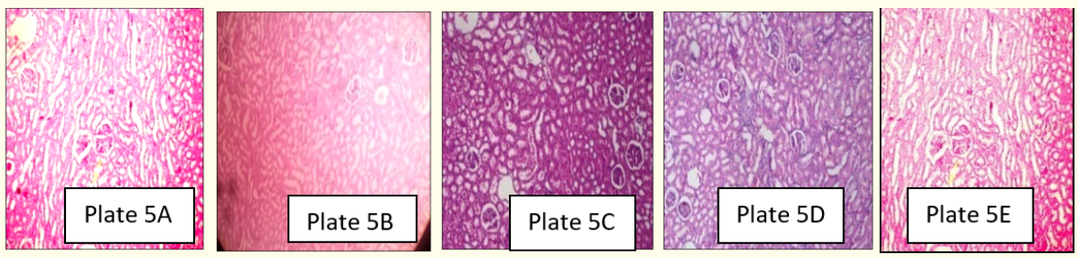

Plate 5: *5A: Photomicrograph of Kidney sections of NC rats shows normal structure of the kidney. 5B:HF-DC group rat demonstrated congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration. 5C: MET group kidney shows congestion of glomerular blood vessels, less hemorrhage, less tubular necrosis, inflammation and focal area as compare to HF-DC group. 5D: VIL group kidney shows congestion of glomerular blood vessels, inflammation and focal area. 5E: BER group kidney shows no congestion of glomerular blood vessels, less tubular necrosis, inflammation.*

Immunohistochemistry of Pancreas for Insulin Localization: Immunohistochemistry of NC group pancreas showed increased localization of insulin in the NC as compared to HF-DC. The HF-DC group showed loss of beta cell mass resulting in decrease in insulin secretion. Treatment groups increased the proportion of beta cell which was functional and secreting insulin as compared to HF-DC. The quantitative immunohistochemical finding of insulin localization within parenchymal cells of pancreas, the NC group rats showed more than 85% positivity. In the HF-DC group < 10% positivity, MET 10 - 12% positivity, VIL 15 - 20% positivity and BER15-20% positivity was noted (Plate 6A, 6B, 6C, 6D and 6E) (40X) (Table 4).

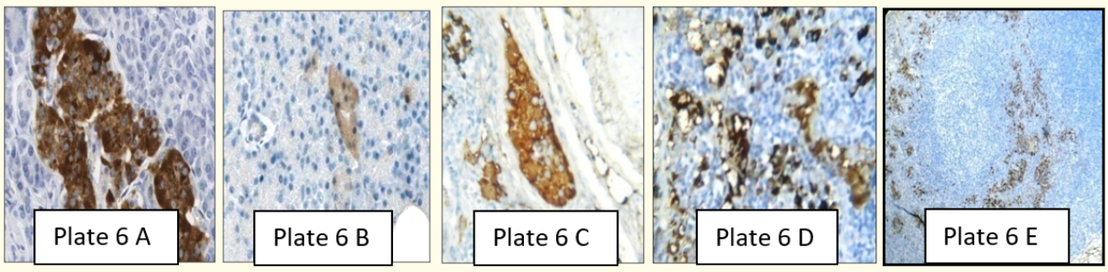

Plate 6: *6A: Immunohistochemistry of NC group pancreas showed increased localization of insulin. 6B: HF-DC group shows decreased insulin localization and hence loss of beta cell functions.6C: MET group pancreas shows increased localization of insulin as compared to HF-DC group.6D: VIL group pancreas shows increased localization of insulin as compared to HF-DC group. 6E: BER group pancreas shows marked increased localization of insulin as compared to standard groups.*

SN	Groups of Rat	IHC- Finding for insulin
1	NC	> 85% positivity is noted within parenchymal cells
2	HF-DC	< 10% positivity is noted within parenchymal cells
3	MET	10 - 12% positivity is noted within parenchymal cells.
4	VIL	15 - 20% positivity is noted within parenchymal cells.
5	BER	15 - 20% positivity is noted within parenchymal cells.

Table 4: *Shows Immnunohistochemistry of pancreas for localization of insulin.*

Discussion

In the present study, the effect of Berberine was assessed using a indigenously developed high-fat diet and STZ-induced diabetes co-existing with metabolic syndrome rat model. Berberine has been used therapeutically to treat a variety of human diseases in Korea, China, and possibly other Asian countries that practice the use of traditional medicines. Berberine has been shown to offer benefits in the management of factors of metabolic syndrome in many ways. The present study demonstrated for the first time that Berberine mitigates diabetes and metabolic syndrome induced changes in experimental rats by favorably modulating diabetic (Blood glucose, HbA1c, restoration of pancreatic function), central obesity (Body weight, AC/TC ratio) and hypolipidemic (favorable lipid profile, atherogenic index) and cardioprotective (CPK-MB) parameters. Also to understand the mechanisms; DPP-IV pathway (serum DPP-IV), anti-inflammatory (hs-CRP levels), antioxidant (MDA) a contributing to the beneficial effects of Berberine in diabetes with metabolic syndrome was studied. In addition, safety parameters {pancreas [lipase (U/L)], liver [SGPT (U/L)], and renal [creatinine (mg/dl)} were also assessed.

Hyperglycemia

The present study evaluated several metabolic parameters such as blood glucose, glycosylated hemoglobin (HbA1c), serum insulin, and C-Peptide. The blood glucose levels in the HF-DC group rats were significantly higher as compared to NC group rats at 4th, 7th, and 10th week. Treatment with Berberine and standard drugs: Metformin and Vildagliptin resulted in a significant reduction in the blood glucose level in comparison to HF-DC group respectively. Clinical finding by Yin j., *et al*. (2008) confirmed that administration of Berberine at the beginning of each major meal was able to reduce fasting blood glucose as well as post prandial blood glucose and HbA1c in adult patients with newly-diagnosed type II diabetes, which was found to be comparable to that of Metformin [24]. The observed hypoglycemic effects of Berberine are also supported by the results of previous *in vitro* and *in vivo* [14,25,26] studies. In addition to hyperglycemia, the present study results also found poor glycemic control as indicated by increased HbA1c levels in HF-DC group as compared with NC. However, the potential of Berberine to reduce glycosylated hemoglobin has not been studied in the setting of diabetes co-existing with metabolic syndrome model.

A deficiency of insulin and a decline in pancreatic function were evidenced by a reduced level of serum insulin, C-peptide, HOMA-beta (marker of beta function) and increased HOMA–IR (marker of insulin resistance) respectively, in HF-DC group as compared to NC group rat. In the present study, treatment with Berberine restored the disturbed glucose homeostasis and improved insulin sensitivity as indicated by the HOMA-IR and β-cell function, indicating that it can improve insulin resistance. Y Wang., et al. (2009) supported insulinotropic capabilities of Berberine [27]. The HOMA index is a well validated surrogate measure of insulin resistance, and a strong predictor of CV risk in several classes of patients C-peptide is considered as an important component in the biosynthesis of insulin and is an excellent parameter for evaluating pancreatic ß-cells function. C-peptide was restored by treatment with Metformin, Vildagliptin and Berberine. In addition, the biochemical results showed restoring effects in blood glucose with concomitant improvement in insulin and C-peptide levels in conformity with histopathological and immune-histochemical findings of Berberine and standard drugs Metformin and Vildagliptin. Berberine treatment restored beta cell mass and function. Not only was the beta cell mass preserved, the cells were functional, viable and were actively secreting insulin.

Central obesity

Various anthropometric parameters such as body weight, and ratios (AC/TC) were evaluated in the Normal control (NC), High fat diabetic control (HF-DC) and treatment groups. Challenge with streptozotocin caused decrease in body weight which is typically seen in type II diabetes but not metabolic syndrome. Berberine supplementation showed no significant effect on body weight. This findings are in agreement with an earlier study report by Y Wang., *et al* (2009). In contrast, significant weight loss was observed with Metformin and Vildagliptin treatment. These results are in conformity with previous reports of Metformin and Vildagliptin on weight loss.

Dyslipidemia

In the present study, HFD/STZ treated rats exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C levels in HF-DC group rats. Treatment with Berberine and standard drugs significantly restored the elevated lipid parameter. In 2004, Kong., *et al.* [28] defined BBR as "a new cholesterol-lowering drug". They demonstrated *in vitro* and *in vivo* the efficacy of this substance in lipid lowering, which was comparable to that of statins. Two clinical trials demonstrated that berberine was able to decrease triglycerides by 35% and 22%, serum cholesterol by 29% and 16%, and LDL-C by 25% and 20% in subjects with dyslipidemia [12]. In animal studies, berberine was shown to decrease triglycerides, serum cholesterol and LDL-C markedly in hamster or diabetic rats fed with high-cholesterol diet [29]. These findings are in agreement with earlier studies.

Cardiac variable

The time course of changes in CPK-MB suggests that the deleterious cardiovascular changes are slow but progressive in nature. CK-MB is a cardiac enzyme and its level in the serum indicates the extent of damage to the cardiac cells. These results are in keeping with our present study, since we found the close relationship between CK-MB and heart tissue. Rajesh Kumar Suman., *et al*. (2016) [30] supported the cardioprotective effects of Berberine in myocardial infarction with diabetes model. However, cardioprotection of Berberine has not been studied so far in the setting of diabetes with metabolic syndrome model. The protective effect of berberine on myocardial injury induced by High Fat Diet and STZ demonstrated by biochemical marker was also confirmed by histopathological assessment.

Mechanism: DPP-IV pathway, inflammatory, oxidant Variables

In the present study, HFD/STZ rats treated with Metformin, Vildagliptin and Berberine showed reduced serum DPP-IV levels in setting of diabetes with metabolic syndrome. The invitro DPPIV inhibitory activity of Berberine translated into significant reduction in serum DPPIV in the experimental model of diabetes with metabolic syndrome. Rituparna Chakrabarti., *et al.* (2011) [31] has reported Berberine to possess DPP-IV inhibitory activity of methanolic extract of the bark of tree turmeric (*Berberis aristata*) with IC50 value of 14.4µg/ml. and Experimentally, berberine was found to inhibit human recombinant DPP IV *in vitro* with IC (50) = 13.3 microM; one of the mechanisms that explain the anti-hyperglycemic activity of berberine.

Berberine showed favorable effect on inflammatory marker: hs-CRP. It is reported that in insulin-resistant states of obesity and T2DM, the plasma concentration of TNF-α is increased [32,33]. In the present study, HFD/STZ treated rats resulted in a significant elevation in plasma levels of the inflammatory biomarkers hs-CRP. Berberine treatment reduced plasmahs-CRP, suggesting the improved ability of Berberine to control blood glucose might be related to its anti-inflammatory and FFA-lowering effects.

Oxidative stress associated with overproduction of reactive oxygen species plays an important role in the development of diabetic complications. In our earlier study, we found that Berberine significantly reduced malonaldehyde (MDA) level, a marker of lipid peroxidation in different organs viz., heart, liver and kidney by ameliorating changes in the antioxidant enzymes indicating its possible antioxidant activity which is advantageous in treatment of diabetic complications. The inhibitory effect of Berberine on oxidative stress was observed both in cells cultured with high glucose-containing media [34]. As inflammation and ensuing oxidative stress forms a critical part of metabolic syndrome and diabetes, the observed effects may contribute to the overall beneficial effects of Berberine.

Safety variable

Several studies have recently showed an impairment of pancreatic exocrine function in type 1 and type 2 diabetes. The analysis of serum/plasma pancreatic enzymes was suggested to provide additional informative parameters for the assessment of the chronicity and progress of the illness as well as of the response to therapy. Increased lipase levels as seen in HFD/STZ rats showed presence of pancreatic tissue damage which was altered by Metformin, Vildagliptin and Berberine monotherapy thereby restoring the architecture of the pancreas. Berberine may significantly promote GLP-1 secretion, thus promoting insulin secretion and improving the function of β-cells in the pancreas [25,35,36]. This is the first report that of the effect of Berberine on the pancreatic function in the experimental model of diabetes coexisting with metabolic syndrome. Chronic treatment with Berberine did not adversely affect the liver and renal dysfunction in diabetic and metabolic syndrome rats, as evidenced hepatic and renal function biochemical markers as well as histopathological studies.

Conclusion

Berberine mitigates diabetes with metabolic syndrome induced deleterious effects. The DPP-IV Inhibitory, hypoglycemic, hypolipidemic, antioxidant, cardioprotective and anti-inflammatory properties of Berberine may contribute to its beneficial effects. Berberine has the potential to be developed as an indigenous natural DPPIV Inhibitor. Efficacy of berberine is comparable to the available synthetic DPPIV Inhibitor: Vildagliptin.

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