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Original Research Article

Metformin ameliorates diabetes with metabolic syndrome induced changes in experimental rats

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Abstract

Objective: Metformin is the primary glucose- lowering agent used in the treatment of type II diabetes mellitus. However, there is no experimental evidence presently available with regard to the possible beneficial effects of Metformin on metabolic syndrome co-existing with diabetes in experimental rats**.** Thus, the present study was designed to evaluate potential effects of Metformin on various components of metabolic syndrome. Also to elucidate the underlying mechanisms: DPP-IV, anti-inflammatory, antioxidant pathways.

Material and 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 wt). Metformin (100 mg/kg) was administered to rat from 4^{th} to 10^{th} 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.

Results: Metformin treatment ameliorated the deleterious effects associated with metabolic syndrome and diabetes. Metformin favorably modulated various parameters: 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 (favorable lipid profile, artherogenic index). In addition significant restoration of cardiac injury as indicated by CPK-MB levels were observed. In addition, DPP-IV pathway (reduced serum DPP-IV), anti-inflammatory (reduced hs-CRP levels), antioxidant (reduced MDA) contributed to the beneficial effects of Metformin in diabetes with metabolic syndrome. The histopathological assessment confirmed the protective effects of Metformin on heart, thoracic aorta, pancreas, liver and kidney.

Conclusion: Metformin treatment ameliorated the deleterious effects associated metabolic syndrome in the setting of diabetes mellitus. Beneficial effects of Metformin can be attributed to hypoglycemic, hypolipidemic, antioxidant, cardioprotective and anti-inflammatory effects.

Keywords: Metformin, Diabetes, Metabolic Syndrome, High Fat Diet, Streptozotocin.

1. 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]. Therefore the co-existence of diabetes with metabolic syndrome is a unique pathology that needs to be addressed as a separate entity.

Metformin an insulin-sensitizing biguanide, exerts an anti-hyperglycemic effect, with minimal risk of hypoglycemia, and has been used to prevent type II diabetes with a 31% reduction in incidence [2]. The anti-diabetic effect of Metformin owes to its ability to suppress hepatic glucose production, enhance peripheral glucose uptake and improve peripheral insulin sensitivity [3-5]. In fact, Metformin showed beneficial effects in type II diabetes, including weight reduction, improved lipid profiles, and enhanced endothelial function. There are evidences for a potential role of Metformin in the prevention of type II diabetes in obese

patients [6]. Several studies have suggested that Metformin may improve some of the features of the metabolic syndrome as it not only improves insulin sensitivity in the liver and muscle, as its primary anti-hyperglycemic mechanism of action, but also induces additional beneficial effects on several metabolic abnormalities associated with the metabolic syndrome [7-9]. Recent studies also reported that Metformin induced improvement of metabolic disorders is associated with the energy state of the body, reduction of macrovascular morbidity and mortality, anti-artherogenic, anti-inflammatory and antioxidant effects [10]. In combination with lifestyle modifications, Metformin is the primary glucose- lowering agent used in the treatment of type II diabetes mellitus, due to its efficacy, safety and beneficial cardiovascular and meta-bolic activity. However, there is no experimental evidence presently available with regard to the mitigating effects of Metformin on diabetes with metabolic syndrome (HFD-STZ induced). Also the potential of Metformin to modulate the DPP-IV Pathway has not been studied so far. The DPP-IV is novel adipokine and to characterize the association of DPP-IV to different parameters of metabolic syndrome and that may impair insulin sensitivity in an autocrine and paracrine fashion, DPP-IV release strongly correlates with adipocyte size, potentially representing an important source of DPP-IV in obesity linking with metabolic syndrome[11].

The present study was designed to evaluate potential effects of Metformin on various components of metabolic syndrome (Blood glucose, HbA1c, Seum 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), cardioprotective 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.

2. Material and Methods

2.1 Chemicals and drugs:

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

2.2 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*.

2.3 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) [12].

2.4 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), Systolic Blood pressure (>130 mm/hg) and reduced HDL levels (<35mg/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 histtopathological evaluation of injury to the Heart, Thoracic aorta, Pancreas, Liver and Kidney.

2.5 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 wt, i.p. dissolved in 0.01 M citrate buffer, pH 4.5).

Group 2: 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 0.01 M citrate buffer, pH 4.5). The Metformin (100 mg/kg) was fed orally to rat from 4th weeks to 10th weeks daily.

2.6 Evaluation parameters

2.6.1 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.

2.6.2 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.

2.6.3 Histopathological studies: At the end of the experiment (10 weeks), the animals were sacrificed. The heart, thoracic aorta, 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 µm 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.

2.7 Statistical Analysis

The Data were analyzed by One –way analysis of variance (ANOVA) and Values were considered at P<0.05.

3. Results

3.1 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 diabetes with metabolic syndrome.

3.2 Anthropometric parameter

The HF-DC and Metformin 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 Metformin group rats was not sustained till the end of 10th week. Metformin (100 mg/kg) treated group rats at week 10, showed significant (p<0.01) lower body weight compared to HF-DC group rats. The weight difference between NC and HF-DC on Baseline weight and 10th week weight was found to be 50.91% in NC, 58% in HF-DC.and 32.86 % Metformin. Similarly, the AC and TC of the Metformin 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 of NC, HF-DC and Metformin group rats. (Table 1)

Table 1: Anthropometric Parameter

SN	Groups	Duration	Variable			
			Body weight	**AC**	**TC**	**AC/TC**
1	**NC**	**Baseline**	157.63±7.11	14.13±0.49	13.06±00.40	1.081
2	**HF-DC**		161.14±5.11	14.28±0.39	13.14±0.55	1.08
3	**Metformin**		159.28±10.48	14.07±0.45	13.00±0.57	1.08
1	**NC**	**4 weeks**	188.87±6.22	15.00±0.26	13.93±0.41	1.076
2	**HF-DC**		235.14±4.59***	17.72±0.48**	16.71±0.48**	1.06
3	**Metformin**		230.57±10.59	17.42±0.93	16.35±1.02	1.06
1	**NC**	**7 weeks**	214.12±5.33	16.31±0.25	15.25±0.26	1.069
2	**HF-DC**		226.42±4.68*	17.00±0.40*	16.00±0.41*	1.06
3	**Metformin**		213.71±11.84$^{\$}$	15.57 ± 0.73$^{\$}$	14.47± 0.73$^{\$}$	1.07
1	NC	**10 weeks**	237.88±4.99	17.68±0.70	16.62±0.74	1.063
2	**HF-DC**		219.14±9.92**	16.58±0.45*	15.57±0.47*	1.06
3	**Metformin**		192.14±10.99$^{\$\$}$	15.35±0.62$^{\$}$	14.35±0.62$^{\$}$	1.06
Weight Differences between Baseline (To) and Final (T 10th week)						
		Weight Baseline (T_0 wk)	**Weight Final (T_{10th} wk)**	**% Difference in weight (T_{10th} wk- T_0 wk/ T_0)**		
NC		157.63	237.88	50.91		
HF-DC		161.14	219.14	58		
Metformin		159.28	192.14	32.86		

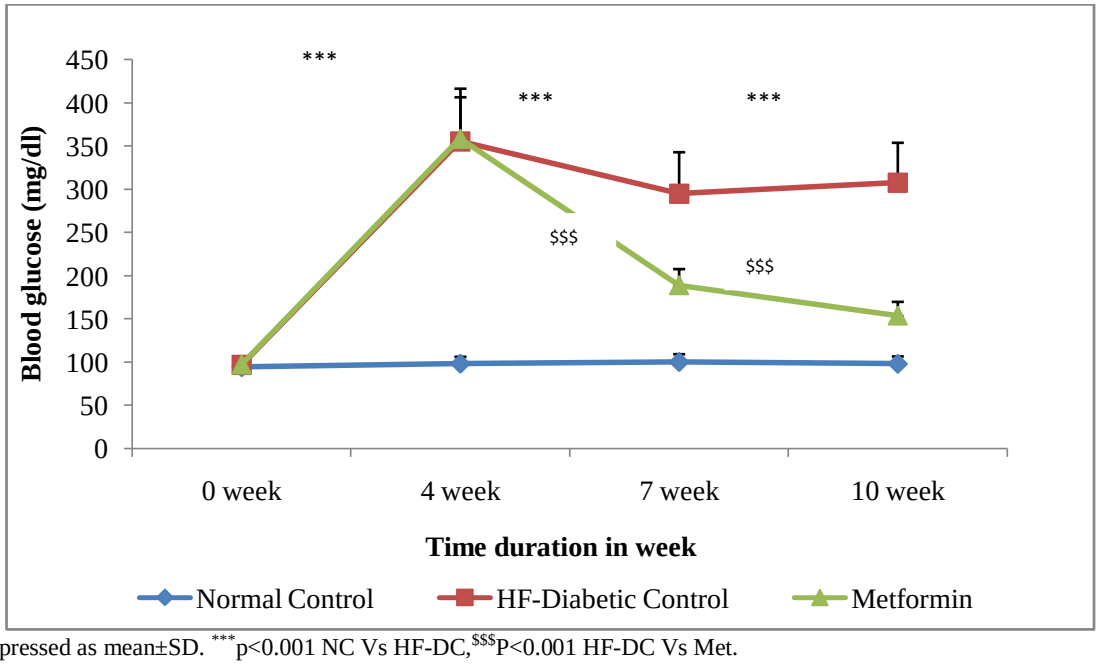
NC: Normal Control group (n=8), **HF-DC:** High fat diabetic control group (n=7) and **Met**: Metformin group (n=8). Values are expressed as mean ± SD. *P<0.05, **p<0.01 NC Vs HF-DC,$^{\$}$P<0.05, $^{\$\$}$p<0.01HF-DC Vs Met.

3.3 Biochemical Parameters:

3.3.1 Metabolic Parameters

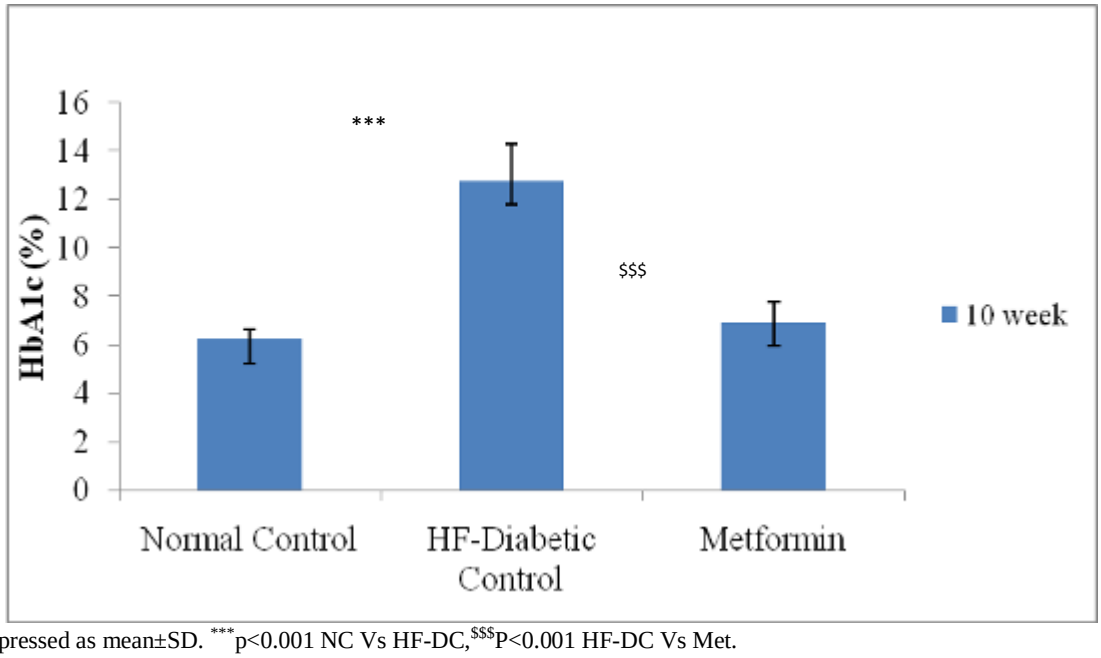
The Blood glucose, Triglyceride and Total Cholesterol levels in the HF-DC group rats were significantly higher ($p<0.001$) as compared to NC group rats. In Metformin group rats these parameters were significantly lower ($p<0.001$) as compared to HF-DC group rats at 7th and 10th week. Glycosylated hemoglobin ($p<0,001$), Total cholesterol ($p<0,001$), Triglyceride ($p<0.01$), Low density lipoprotein ($p<0,001$), HOM-IR, and atherogenic index ($p<0.01$) were significantly reduced in Metformin group. Serum insulin ($p<0.01$), and HOMA-β ($p<0.01$) was significantly increased in Metformin group as compared with HF-DC group at the end of 10th weeks. High density lipoprotein was significantly ($p<0.05$) increased in metformin group rats as compared with HF-DC. The C-peptide levels in Metformin group increased though statistically not significant as compared to NC and HF-DC group rats. (Figure 1,2,3 and Table 2,3)

Figure 1: Time course changes of Blood Glucose level of NC (n=8), HF-DC group (n=7) and Met group (n=8).



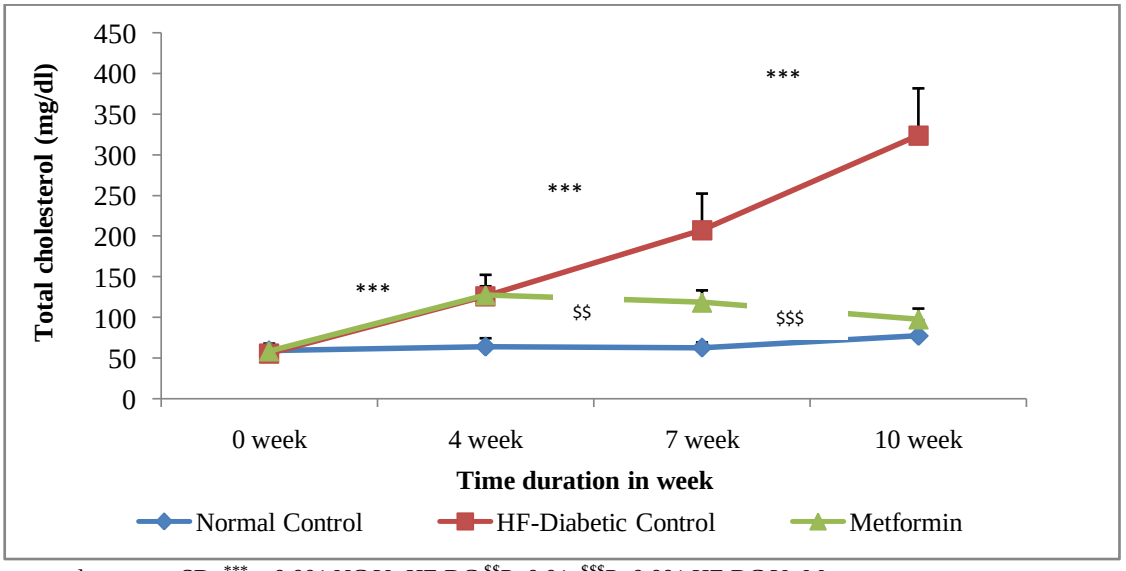
Values are expressed as mean±SD. ***p<0.001 NC Vs HF-DC,$$$P<0.001 HF-DC Vs Met.

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



Values are expressed as mean±SD. ***p<0.001 NC Vs HF-DC,$$$P<0.001 HF-DC Vs Met.

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



Values are expressed as mean±SD. ***p<0.001 NC Vs HF-DC,$^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001 HF-DC Vs Met.

Table: 2: Assessment of insulin resistance parameter in various experimental Groups

SN	Name of Parameter	Insulin	C-Peptide	HOMA IR	HOMB-β
1	NC	6.46±0.65	0.07±0.02	1.57±0.16	66.6±5.86
2	HF-DC	2.93±1.11**	0.05±0.03	2.17±0.63**	5.9±2.2$^{\$\$}$
3	Metformin	4.33±1.2$^{\$\$}$	0.073±0.02	1.64±0.5	17.2±4.29$^{\$\$}$

NC: Normal Control group (n=8), **HF-DC:** High fat diabetic control group (n=7) and **Met**: Metformin group (n=8). Values are expressed as mean ± SD. **p<0.01 NC Vs HF-DC, $^{\$\$}$p<0.01HF-DC Vs Met.

Table 3: Lipid profile in various experimental Groups

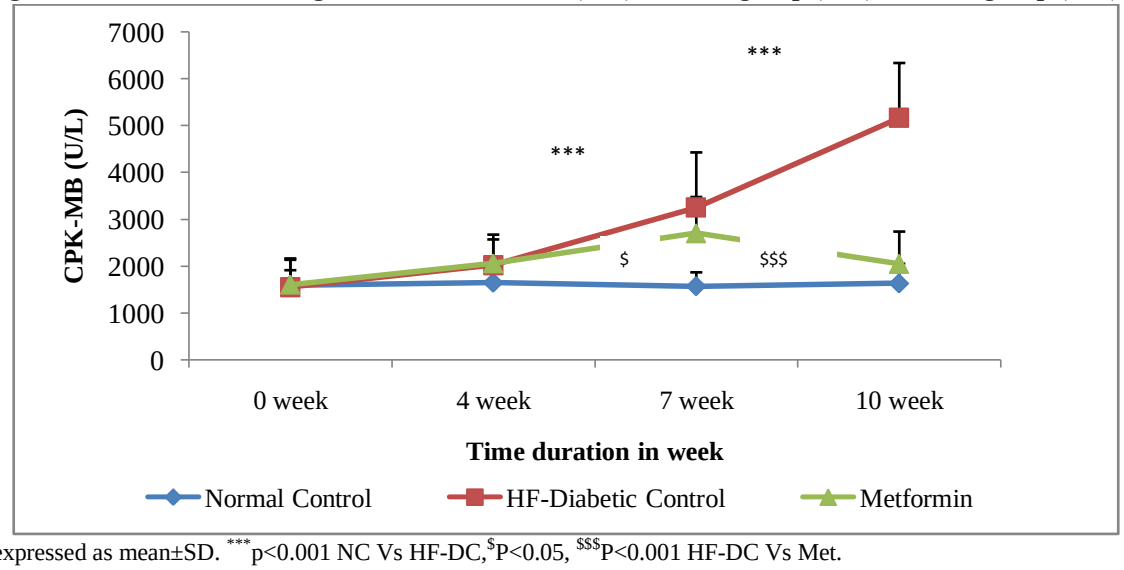
SN	Variable	NC	HF-DC	Metformin
1	TG (mg/dl)	63.75±11.47	312.85±62.24**	174.14±111.82$^{\$\$}$
2	HDL (mg/dl)	32.62±2.56	26.57±5.74*	32.71± 5.05$^{\$}$
3	LDL (mg/dl)	12.6±2.41	62.57±12.44***	34.82±22.36$^{\$\$\$}$
4	Atherogenic Index	1.36±0.20	11.97±4.76**	2.03±0.58$^{\$\$}$

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

3.3.2 Cardiac Variables

The CPK-MB levels of 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 Metformin group rats at 7th and 10th week as compared with HF-DC. (Figure 4)

Figure 4: Time Course Changes in CPK-MB of NC (n=8), HF-DC group (n=7) and Met group (n=8). .



Values are expressed as mean±SD. ***p<0.001 NC Vs HF-DC,$^{\$}$P<0.05, $^{\$\$\$}$P<0.001 HF-DC Vs Met.

3.3.3 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. Metformin treated rats showed significant reduction in serum DPP-IV level as compared to HF-DC rats. Similarly inflammatory (hs-CRP) ($p<0.05$), oxidative marker (MDA) ($p<0.01$) also significantly reduced in Metformin group as compared to HF-DC group rats on 10th week. Hence it shows restoring effects in treatment groups. (Table 4)

Table 4: Mechanism; DPP-IV pathway, inflammatory, oxidant Variables in various experimental Groups

SN	Name of Parameter	NC	HF-DC	Metformin
1	Serum DPP-IV (microunit/ml)	4.76±0.48	44.53±5.04***	28.45±3.58$^{\$\$}$
2	Hs-CRP (mg/dl)	0.86±0.11	2.2±0.52*	1.11±0.31$^{\$}$
3	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 **Met**: Metformin group (n=8). Values are expressed as mean ± SD. *P<0.05, ***P<0.001 NC Vs HF-DC,$^{\$}$P<0.05, $^{\$\$}$ P<0.01HF-DC Vs Met.

3.3.4 Pancreatic, Liver and kidney function markers

The Metformin group rats showed a significant reduced in the level of pancreatic lipase (U/L) ($p<0.05$), 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 5: Shows Safety marker in various experimental groups

SN	Variable	NC	HF-DC	Metformin
	Pancreatic Marker			
1	Pancreatic Lipase(U/L)	33.66±4.62	48.26±9.36*	36.83±5.50$^{\$}$
	Liver Marker			
2	SGPT(U/L)	62.77±11.58	99.85±10.38***	68.25±6.60$^{\$\$}$
	Kidney Marker			
3	Creatinine (mg/dl)	0.32±0.07	1.27±0.43***	0.42±0.06$^{\$\$}$

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

3.4 Histopathological assessment

3.4.1 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-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 Metformin 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-Diabetic Control group (Plate 1C). (H&E x 40)

3.4.2 Histopathological section of Aorta.

Photomicrograph of aorta sections of Normal control 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-Diabetic Control group rat 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 Metformin treatment group rats aorta shows, well maintain alignment of aortic layer with intact intima (Plate 2C). (H&E x 40)

3.4.3 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-Diabetic Control group rat shows 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 Metformin treatment group rats Pancreas shows, improve beta cell mass less fibrosis, less inflammatory infiltration and hemorrhage as compared to HF-DC group (Plate 3C). (H&E x 40)

3.4.4 Histopathological section of Liver

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

3.4.5 Histopathological section of Kidney

Photomicrograph of Kidney 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 (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 Metformin 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 5C) (H&E x 40)

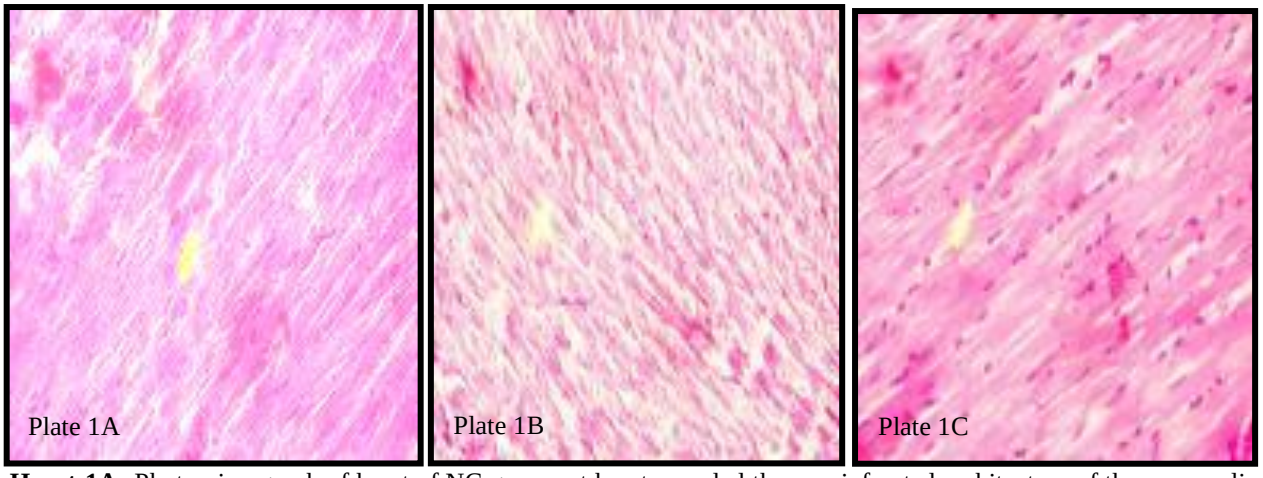


Plate 1:Heart:1A: Photomicrograph of heart of NC group rat heart revealed the non-infracted architecture of the myocardium. **1B:** HF-Diabetic Control group rat heart shows fatty infiltration in myocardial cells, hemorrhage, marked edema, confluent areas of myonecrosis separation of myofibers, congested blood vessels and inflammation. **1C**: In the Metformin treatment group rats, occasional focal myofiber loss and the degree of edema, inflammation and necrosis was less as compared to the HF-Diabetic Control group.

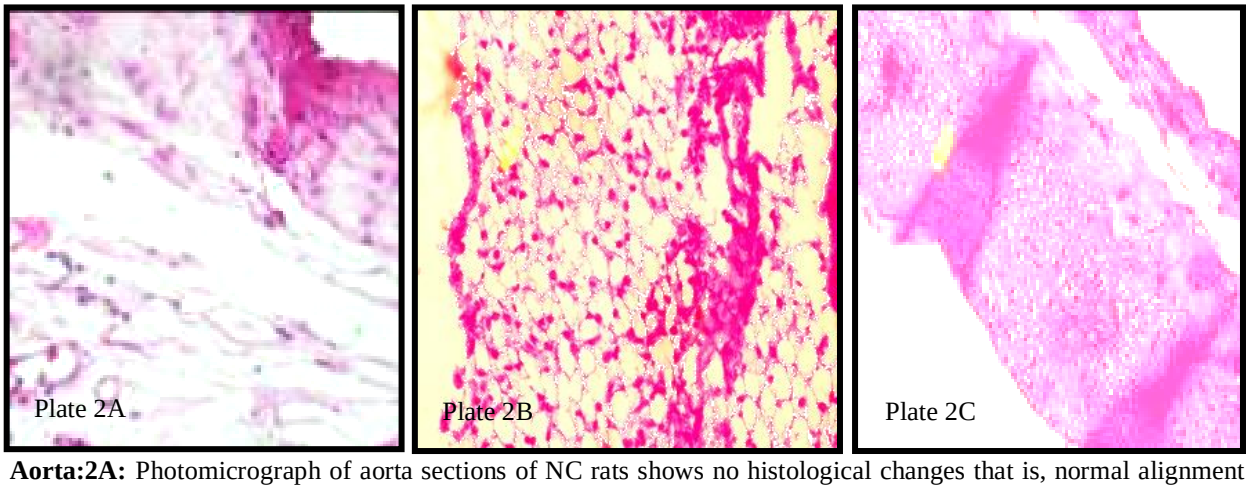


Plate 2: Aorta:2A: Photomicrograph of aorta sections of NC rats shows no histological changes that is, normal alignment of tunica media, tunica intima, and tunica adventitia as seen. **2B:** In case of HF-Diabetic Control group rat aorta shows, focal and vacuolated cells, inflammation, edema, necrosis were seen in aortic layer and atherosclerotic deposition in the vessel wall. **2C:** In the Metformin treatment group rats aorta shows, well maintain alignment of aortic layer with intact intima.

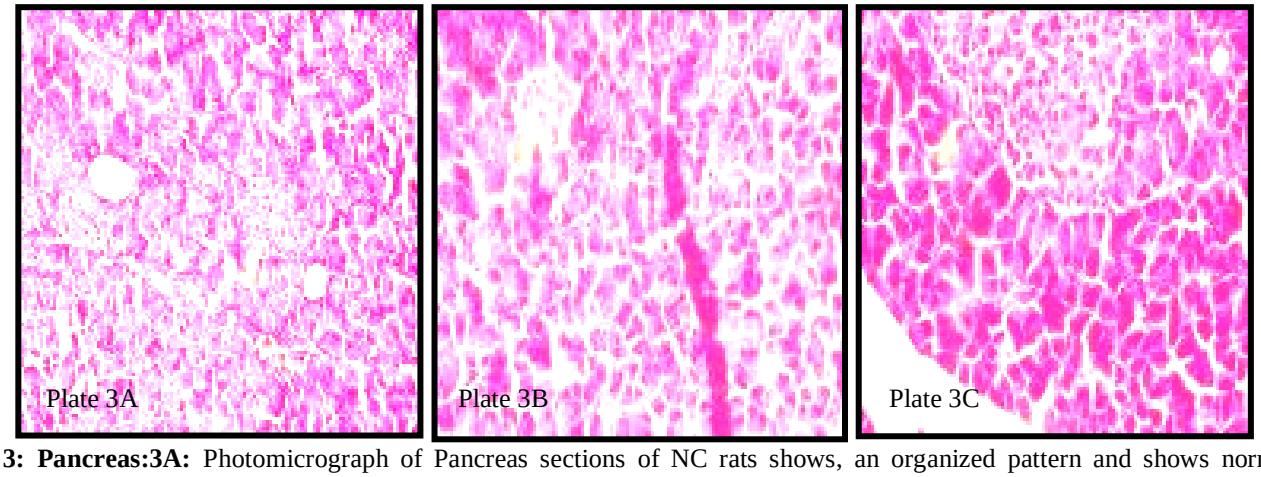


Plate 3: Pancreas:3A: Photomicrograph of Pancreas sections of NC rats shows, an organized pattern and shows normal architecture of islets of langerhans and the beta cells. **3B: T**he Pancreas of HF-Diabetic Control group rat shows severe degenerative changes in the pancreatic islets, damaged islets 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. **3C:** In the Metformin treatment group rats Pancreas shows, improve beta cell mass less fibrosis, less inflammatory infiltration and hemorrhage as compared to HF-DC group.

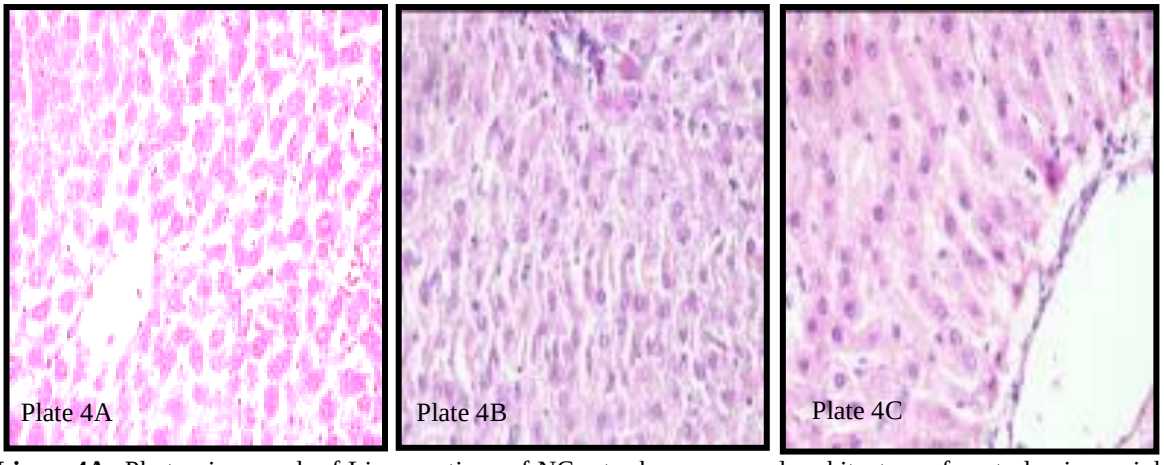


Plate 4 :Liver: 4A: Photomicrograph of Liver sections of NC rats shows, normal architecture of central vein, peripheral vein and no congestion of sinosoides.**4B:** In contrast, the liver of HF-Diabetic Control group rat shows Fatty liver, moderate fatty degeneration, ballooning of cell, inflammatory infiltration more and congestion of blood vessels in central vein.**4C:**In the Metformin treatment group rats Liver shows, less fatty degeneration, less inflammatory infiltration, congestion of blood vessels, fibrosis, edema and necrosis as compared to HF-DC group.

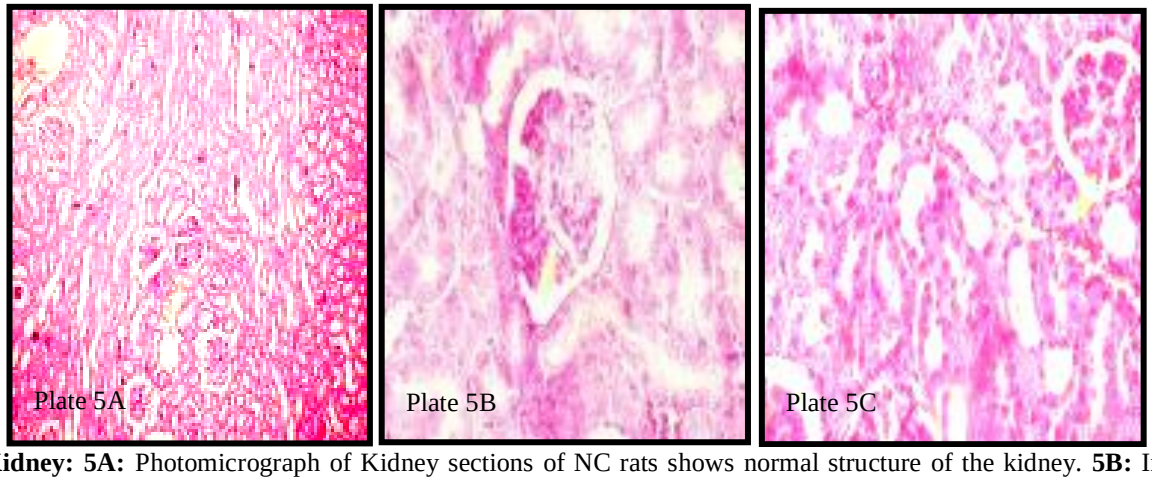


Plate 5:Kidney: 5A: Photomicrograph of Kidney sections of NC rats shows normal structure of the kidney. **5B:** In contrast histological assessment of the HF-DC group rat demonstrated congestion of glomerular blood vessels, tubular necrosis, inflammation and cloudy degeneration as compared to NC group. **5C:** In Metformin treated group kidney shows congestion of glomerular blood vessels, less hemorrhage, less tubular necrosis, inflammation and focal area as compare to HF-DC group.

4. Discussion

Metformin is an oral antidiabetic drug that has been used for decades to reduce plasma glucose, improve insulin sensitivity, increase peripheral glucose uptake, and inhibit hepatic glucose production. Previous studies reported that Metformin could improve some of the features of the metabolic syndrome. Despite these beneficial effects, Metformin's therapeutic effect in the setting of diabetes with metabolic syndrome in experimental rats remains unknown.

The major finding of this study is that Metformin ameliorates diabetes with metabolic syndrome induced deleterious changes in experimental rats. Metformin favorably modulated anti-diabetic (Blood glucose, HbA1c, 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 Metformin in diabetes with metabolic syndrome was studied.

4.1 Essential components of Metabolic Syndrome with diabetes:

4.1.1 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 Metformin treatment at the end of 10 weeks. The previous study by Latha P. *et al.* (2012) [13] showed similar results by significantly reversed the HFD/STZ induced increase in blood glucose levels. The HOMA-IR was reduced whereas HOMA-β was increased in Metformin treated rats. Study by Dieudonne Kuate *et al* (2015) [14] supported our finding. This observation implied that Metformin could exert its hypoglycemic effect through improving peripheral Insulin resistance and protecting

pancreatic islet β-cells and/or stimulating insulin secretion. The C-peptide determined by Nora M. El- Sheikh (2012) [15] showed reduced level of C-peptide in diabetic rats similar to present study which is restored by treatment with Metformin. T2DM is characterized by progressive β cell destruction, as a result of chronic IR and the loss of β cell mass and function, as demonstrated by previous studies [16]. The primary mechanism underlying the reduction in β cell mass in patients with T2DM is the apoptosis of β cells in all diabetic patients [17]. In patients with T2DM, pancreatic β cells are unable to secrete sufficient quantities of insulin to compensate for the reduced insulin sensitivity, which is primarily a result of insulin secretion dysfunction and a marked reduction in the number of functional β cells. Consistent with these findings, the present study observed similar effects, as in the Metformin group rats, restoration of β cell mass was observed as compared to HF-DC group.

4.1.2 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 Metformin groups. The weight difference between NC, HF-DC and Metformin on Baseline weight and 10^{th} week weight are 50.91% in NC, 58% in HF-DC.and 32.86 % Metformin. Like previous investigators, the weight loss during Metformin treatment was significant. Similarly, the anthropometric results may be attributed to the diabetic state that is known to cause weight loss as shown by Dieudonne Kuate *et al* (2015) [14]. Meanwhile, the present results are consistent with the findings of Gad *et al* (2010) who reported weight reduction by Metformin [18].

4.1.3 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. These results are in accordance with previous reports by Siddiqui *et al*. (2011) [19]. Study by Ismail *et al* (2015) showed treatment of Metformin significantly reduced the TC, TG, LDL-C level and increased HDL-C levels in HFD/STZ rats [20]. Metformin 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 High Fat Diet and STZ in rats. However, treatment of Metformin significantly restored increase in serum CPK-MB levels at 7^{th} and 10^{th} week. The study by Arshiya Shamim *et al* (2015) [21] showed increase in the cardiac marker enzymes CPK-MB in diabetic high fat diet rat clearly due to the presence of all potentiating factors i.e., STZ, ISO and HFD. The Myocardial injury induced by High Fat Diet and STZ shown by biochemical marker was also confirmed by histopathological assessment.

4.1.5 Mechanism; DPP-IV pathway, inflammatory, oxidant Variables

Many studies involving DPP- IV inhibitors support a principal role for DPP- IV in the inactivation of GLP-1 in vivo. 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 [22]. In our study HFD/STZ treated with metformin rats showed reduced serum DPP-IV level in setting of diabetes with metabolic syndrome. Yasuda *et al* in 2002 found that Metformin treatment increased the active levels of GLP-1 in rats, but concluded that this was due to metformin increasing GLP-1 secretion and not a direct inhibitory effect on DPP- IV [23]. However, the Lenhard *et al* in 2004 [24] showed that prolonged dosing of Zucker diabetic rats with Metformin reduced serum DPP- IV activity in vivo. They postulated that prolonged Metformin treatment may reduce DPP- IV secretion. Similar results found with Lindsay *et al* in 2005 [25] demonstrated that oral Metformin therapy effectively inhibits DPP-IV activity in patients with type II diabetes.

Similar to the present findings Nicolas F. Renna (2014) [26] also found raised hs-CRP in Fructose fed Hypertensive rats as compared with normal rats. Similar results were shown by present study. Metformin treated significantly reduced in these markers reported by Farnood F1, *et al* (2014) [27]. Lipid peroxidation, is often used as an index of oxidative tissue damage which causes free radical damage to membrane components of the cell and resulting cell necrosis and inflammation [28]. 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. Treatment with Metformin significantly modulated these parameters. Our data are in accordance with the previous report by Kuate *et al* (2015) [14].

4.1.6 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. Metformin treated rats restored the architecture of the pancreas. In the present study HFD/STZ treated rats showed increased levels of SGPT liver enzymes. Numerous studies have reported that diabetes is associated with raised levels of SGPT. In a large clinical study reported by Erbey JR *et al*., (2000) [29] patients who were overweight (BMI 25–30 kg/m2) and obese (BMI > 30 kg/m2) were more likely to have elevated SGPT levels. There was 10.6% prevalence in obese diabetic patients versus 6.6% prevalence in obese non diabetic patients. Supplementation of Metformin ameliorated

all these changes. Latha *et al*.(2012) [13] demonstrated similar results by reducing SGPT level in High Fat Diet/ STZ induced obese diabetic rats. In addition, recent evidence suggests that diabetic condition is associated with changes in morphology and eventually functional alteration in kidneys. The present results clearly demonstrate increased levels of kidney function marker creatinine in serum of HFD/STZ group. In contrast, the HFD/STZ Metformin treated rats showed significant reduction in these markers, thus showing its ability to protect against high fat diet diabetes-induced kidney damage. Similar were the findings showed by Kuate *et al* (2015) [14]. The present study also confirmed the protective effects of Metformin on pancreases, liver and renal function as shown by biochemical findings and histopathological assessment of pancreases, liver and kidney.

The study results have demonstrated the beneficial effects of Metformin (100 mg/kg) on deleterious changes induced by Diabetes with metabolic syndrome via multiple mechanisms: hypoglycemic, hypolipidemic, antioxidant, cardioprotective, anti-inflammatory and DPP-IV Inhibitory property.

5. Conclusion

In summary, Metformin treatment ameliorated deleterious effects associated with Diabetes and metabolic syndrome. The hypoglycemic, hypolipidemic, antioxidant, cardioprotective, anti-inflammatory and DPP-IV Inhibitory properties of Metformin may contribute to its beneficial effects. Metformin was also found to be safe the heart, pancreas, liver and kidney. Our findings provide a lead for the further evaluation of Metformin for the treatment of Diabetic patients with metabolic syndrome.

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