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Research Article

Metformin ameliorates diabetic cardiomyopathy in adult male albino rats in type 2 diabetes

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Abstract

Background: Metformin is the first-line treatment for T2DM especially in obese or overweight diabetic patients. The aim of the present study was to evaluate the effect of metformin monotherapy on body weight, glycaemic control, lipid profile, oxidative stress and cardiac injury in type 2 diabetic rats. **Methods:** Rats were classified into Three groups; control, diabetic and Diabetes + Metformin. Diabetes was induced by receiving high-fat diet for 2 weeks followed by intraperitoneal injection of streptozotocin**.** Metformin was given orally for 28 days after confirmation of diabetes. Serum glucose, insulin, lipid profile, cardiac injury markers and oxidative stress markers (MDA and TAC) were measured and heart histopathology was done. **Results:** The diabetic group showed significantly elevated serum levels of glucose, cardiac injury markers and MDA with disrupted lipid profile along with histopathological features of cardiac tissue injury and significantly decreased serum levels of insulin and TAC in comparison with control group. Treatment with metformin produced significant decrease in both food intake and body weight and significantly decreased serum levels of glucose, cardiac injury markers and MDA, besides significantly elevated serum levels of insulin and TAC with improvement of the lipid profile and the histopathological picture in comparison with the diabetic group. **Conclusion**: Metformin produces beneficial changes regarding weight, food intake, glycaemic control, hypoinsulinemia, hyperlipidemia and cardiac and oxidative injury along with improved cardiac histopathological picture.

Keywords: Diabetic cardiomyopathy; Metformin; Oxidative stress

Introduction

Diabetes mellitus (DM) is one of the most common endocrine disorders. It is considered a group of chronic metabolic diseases which is characterized by high blood sugar due to inability of the pancreas to produce enough insulin or the body cells do not effectively use or respond to the insulin. It is classified into type 1 diabetes mellitus (T1MD) and type 2 diabetes mellitus (T2DM). T2DM is the most common type which represents about 85% to 95% of diabetic cases[(1)].

Diabetes mellitus especially T2DM leads to macrovascular complications such as cardio-vascular disease (CVD) and microvascular complications which affect the kidney, the retina and the nervous system. In T2DM, CVD develops earlier and with greater severity, than in individuals without diabetes mellitus independent of age, smoking status, BMI and systolic blood pressure[(2)]**.** CVD accounts a major cause of morbidity and mortality in diabetic patients. One of CVDs is diabetic cardiomyopathy which is defined as ventricular dysfunction that occurs independently of hypertension or myocardial ischemia in diabetic individuals [(3)].

Metformin (N, N-dimethylbiguanide) is an anti-diabetic drug that is taken orally to reduce blood sugar[(4)]. It is the first-line treatment for

T2DM especially in obese or overweight diabetic patients[5]. It leads to a reduction in macrovascular and microvascular complications in overweight people with newly diagnosed type 2 diabetes[6].

The aim of the present work is to evaluate the effect of T2DM on the heart and oxidative injury and the effect of metformin monotherapy on these diabetic changes.

Materials and Methods

Animals and groups

After exclusion of the dead, the present study was conducted on 30 adult male albino rats, weighing (180-200 gm) at the beginning of this study. They were housed at room temperature with 12:12 hour light/dark cycles for two weeks after arrival from the supplier for acclimatization. Rats were fed a standard diet of commercial rat chow and tap water *adlibitum* until the time of the experiment[7].

Experimental Groups:

Animals were randomly classified into the following groups: (10 rats each):

1- **Control Group (C).**
In which each rat received only vehicle (0.01 M citrate buffer, pH 4.5)[8].

2- **Diabetic Group (D):**
In which each rat received high-fat diet (HFD) containing 18% carbohydrate, 41% protein and 40 % fat to induce T2DM for 2 weeks followed by intraperitoneal injection of streptozotocin at dose 35 mg/kg dissolved in freshly prepared 0.01 M citrate buffer at pH 4.5 [9]. After 3 days from injection, the blood glucose was measured by a blood sample obtained from rat tail. The rat whose blood glucose was ≥ 200 mg/dl was considered diabetic [10].

3- **Diabetic + Metformin Group (D+M)**
In which each rat received high-fat diet (HFD) and streptozotocin as previous group. Then after confirmation of diabetes, each rat received metformin at a dose of 200 mg/kg/day orally for 28 days [11].

Blood sample collection and analyses:

At the end of the experimental period, all rats were sacrificed by decapitation and blood samples from jugular vein were obtained. Blood samples were collected in tubes and left to clot at room temperature_then centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The supernatant serum was then withdrawn into labeled eppendorf tubes and stored at - 20°C for estimation of glucose using glucose oxidase colorimetric kit (Spinreact, Spain), insulin level by enzyme-linked immunesorbent assay kit (ELISA) (Calbiotech,USA), total cholesterol (TC) by using cholesterol oxidase/peroxidase kit, triglycerides (TG), high-density lipoprotein (HDL) using cholesterol HDL direct detergent kit and low-density lipoprotein (LDL) using cholesterol LDL direct detergent kit (BioSystems S.A., Spain). In addition cardiac injury markers; lactate dehydro-genase (LDH), total creatine kinase (CK) level (Spinreact, Spain) and creatine kinase MB isoenzyme (CK-MB) level using creatine kinase-MB (CK-MB) kit (BioSystems, Spain).The levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured using colorimetric assay kit (Biodiagnostic, Egypt)

Result

I. Changes in body weight in the different studied groups during the experimental period.

Effect of HFD feeding on body weight of rats; Data presented in Table (1), illustrates that the mean of the initial body weights (IBW) were not significantly different among all groups, and that HFD feeding for 2 weeks resulted in significantly higher body weight when compared with NPD-fed control rats. As regard the effect of STZ injection on body weight of HFD fed rats; Injection of STZ (35 mg/kg, IP) after 2 weeks of HFD resulted in an insignificant lowering in the body weight during the week of injection (3rd week of dietary manipulation) and it was still significantly higher than the control rats. Regarding the mean of body weight after the 4 weeks of treatment with metformin, it was significantly lower in the D + M group in comparison with control group. (Table 1).

II. Changes in food intake in the different studied groups during the experimental period.

As shown in table (2), the initial food intake during the acclimatization period was insignificant between all groups. HFD feeding showed a significant decrease in food intake as

compared to the control group starting from the first week and continued till the end of the experiment.

Injection of STZ (35mg/kg, IP) after 2 weeks of HFD resulted in further increase in food intake which was significantly higher than the control rats. Administration of metformin produced significant decrease in food intake in D + M group in comparison with D group.

III. The changes in serum levels of glucose and insulin in different experimental groups:

Figure 1 shows significant increase in serum glucose level and significant decrease in serum insulin level in diabetic group in comparison with control group. While, administration of metformin produced significant decrease in serum glucose level and significant increase in serum insulin level in (D + M) group in comparison with (D) group.

IV. The changes in serum lipid profile (total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) in different experimental groups

As shown in table (3), serum levels of total cholesterol, triglyceride and LDL were increased significantly, but serum HDL level was significantly decreased in diabetic group in comparison with control group. While, administration of metformin produced significant decrease in serum levels of total cholesterol, triglyceride and LDL and significantly increase in serum HDL level in (D + M) group in comparison with (D) group.

V. The changes in serum levels of cardiac injury markers (lactate dehydrogenase (LDH), total creatine kinase (CK) and creatine kinase MB isoenzyme (CK- MB) and oxidative markers (malondialdhyde (MDA) and total antioxidant (TAC) in different experimental groups:

Data presented in table (4), show that serum levels of LDH, CK, CK- MB and MDA were significantly increased and TAC was significantly decreased in diabetic group in comparison with control group. While, administration of metformin produced significant decrease in serum LDH, CK and CK- MB and MDA levels and significant increase in TAC in (D + M) group in comparison with (D) group.

Table (1): Body weight changes in the different studied groups

Time	Groups			Sig
	Control (C) on normal diet (NPD)	Diabetic rat on high fat diet (HFD)		
		D	D+ M	
IBW(g)	190.8 ±4.9	188.3±8.7	187.5±12.6	NS
After 2weeks	213.7^{b} ±3.5	277.8^{a} ±5	270.7^{a} ±5.3	**
Streptozotocin (STZ) injection				
One week after STZ	227.3^{b} ±2.2	265.7^{a} ±5.7	259.3^{a} ±6.2	**
Start of treatment for 4 weeks				
Final B.W.	252.3^{b}±2.3	256.2^{a}±2.4	229^{c} ±2.7	**

Means in the same horizontal row with different superscripts $^{a, b \text{ and } c}$ are significantly different ($P < 0.05$). NS: not significant. * $P < 0.05$. ** $P < 0.01$ D: diabetic group, D + M: diabetic + metformin group. IBW: Initial body weight, sig : significance. Data are expressed as M ± S.E.M of 6 rats in each group.

Table (2): changes in food intake in the different studied groups during the experimental period

Time	Groups			SIG
	Control (C) on normal diet (NPD)	Diabetic rat on high fat diet (HFD)		
		D	D+M	
Initial food intake (g/day)	87.3±1.5	85.7±1.3	86.7±1.2	**NS**
1st week	86.2[a]±1.4	66.7[b]±1.6	68.1[b]±0.8	**
2nd week	87.2[a]±1.2	64.5[b]±1.4	63.47[b]±0.72	**
Streptozotocin (STZ) injection				
3rd week	83.3[b]±0.67	87.3[a]±0.84	86.6[a]±0.87	**
Start of treatment for 4 weeks				
4th week (Start of TTT)	82.8[b]±0.7	87.02[a]±0.7	63.1[c]±0.90	**
5th week	83.8[b]±0.7	87.5[a]±0.51	61.47[c]±0.84	**
6th week	84.4[b]±0.66	88[a]±0.5	60.15[c]±0.7	**
7th week (End of TTT)	84.7[b]±0.56	88.8[a]±0.3	58.8[c]±0.87	**

Data are expressed as mean ± SE of 10 rats in each group. [a, b and c] Means in the same horizontal row with different superscripts are significantly different. D: diabetic group, D + M: diabetic + metformin group.

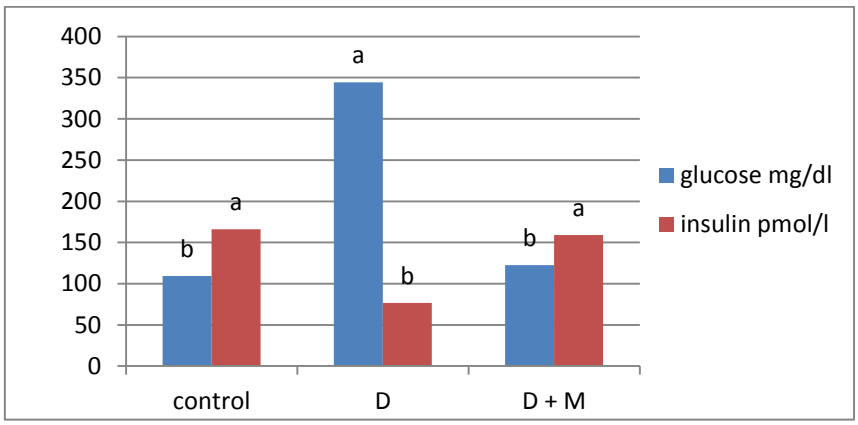

Figure (1): Shows serum glucose and insulin levels different experimental groups

Table (3): Shows the changes in serum lipid profiles (total cholesterol, triglyceride, HDL and LDL) in different experimental groups

Parameters	Groups		
	Control	D	D + M
Total cholesterol (mg/dl)	88.5 ± 3.6[b]	371.5 ± 9.66[a]	104.5 ± 5.49[b]
Triglyceride (mg/dl)	101.1 ± 5.35[b]	392.7 ± 10.21[a]	113 ± 4.84[b]
HDL (mg/dl)	50.5 ± 1.5[a]	21.13 ± 0.631[b]	46.98 ± 1.164[a]
LDL (mg/dl)	25.61 ± 2.234[b]	52.1 ± 2.15[a]	28.91 ± 1.99[b]

Means in the same horizontal row with different superscripts ([aand b]) are significantly different ($P \leq 0.05$). D (diabetic), D + M (diabetic + metformin), HDL (high density lipoprotein) and LDL (low density lipoprotein). Data are expressed as M ± S.E.M of 6 rats in each group.

Table (4): Shows the changes in serum levels of cardiac injury markers (LDH, CK and CK-MB) in different experimental groups

Parameters	Groups		
	Control	D	D + M
Cardiac injury markers			
LDH (mg/dl)	228.5 ± 1.639 [c]	462.8 ± 4.41 [a]	295.45 ± 3.86 [b]
CK (U/l)	103.9 ± 2.208 [c]	360.3 ± 9.135 [a]	188.1 ± 3.046 [b]
CK - MB (U/l)	89.17 ± 0.6[c]	243.6 ± 3.11[a]	150.83 ± 1.7[b]
Oxidative injury markers			
MDA (mg/dl)	0.905 ± 0.01839[b]	3.07 ± 0.1450 [a]	1.036 ± 0.033[b]
TAC (mM/L)	2.08 ± 0.0674[a]	0.898 ± 0.02242 [b]	1.915 ± 0.027 [a]

Means in the same horizontal row with different superscripts ([a, b and c]) are significantly different ($P \leq 0.05$). D (diabetic), D + M (diabetic + metformin), lactate dehydrogenase (LDH), total creatine kinase (CK), MDA (malondialdhyde) and TAC (total antioxidant capacity)creatine kinase MB isoenzyme (CK- MB), MDA (malondialdhyde) and TAC (total antioxidant capacity). Data are expressed as M ± S.E.M of 6 rats in each group.

Histopathology

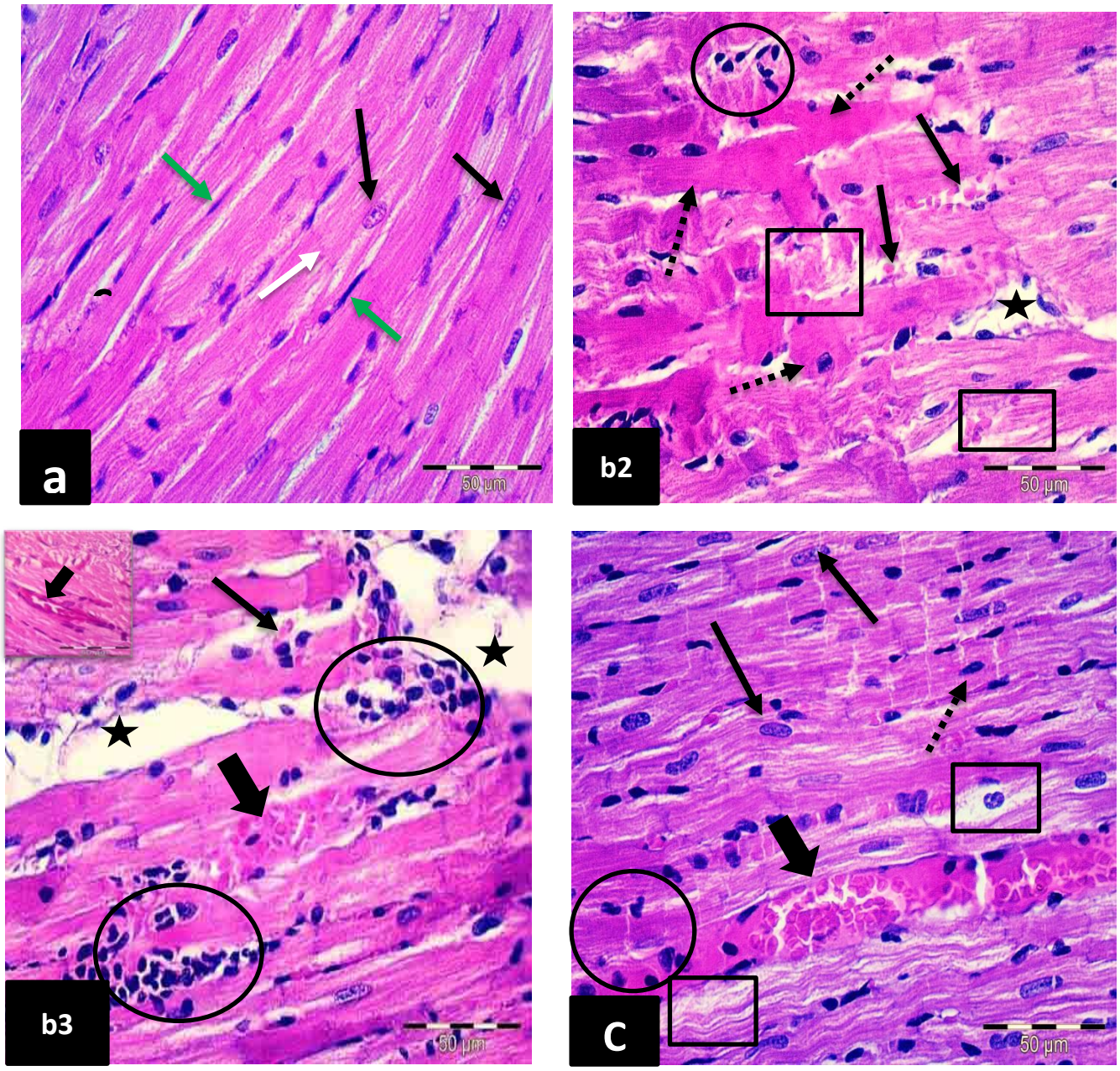

Figure (2): Cardiac histopathology in control group (a); showed branching striated cardiac muscle fibers with central nuclei (black arrows), blood capillaries (c), and intercalated disc (white arrow). elongated nuclei of interstitial cells (green arrows) in the interfiber spaces. **D** group (b1 and b2) showed sever changes in the form of areas of degenerated muscle fibers (squares). Some fibers appear with pyknotic nuclei and hypereosinophilic cytoplasm (dotted arrows), Notice congested blood capillaries (thick black arrow), inflammatory cells infiltration (circles), extravasated RBCs (thin black arrows) and edema in-between muscle fibers (stars). But in D + M group (C), cardiac histopathology showed areas of preserved muscle fiber with central oval nuclei (black arrows) with mild degenerated muscle fibers (squares),.moderate dilated congested capillaries (thick black arrow) with moderate inflammatory cells infiltration (circle), and cells with pyknotic nuclei (dotted arrow).

Discussion

Type 2 diabetes mellitus (T2DM) is considered a complex and progressive disease that is associated with significant morbidity and mortality. It accounts for four million deaths all over the world [12].

The most commonly used animal model of type 2 diabetes is the high fat diet (HFD) followed by streptozotocin (STZ) injection. It accelerates the time-course of type 2 diabetes by initiating a state of obesity-associated insulin resistance during the period of HFD feeding. Then, injection of STZ leads to reduction of pancreatic beta cell mass which occurs as a result of interruption of a number of important cellular processes which finally culminate in DNA damage and cell death [13].

The data of the present study showed significant decrease in food intake and significant increase in body weight during the first 3 weeks of high fat diet in comparison with control group. This occurs as a result of increased satiety produced by high fat diet [14]. While, the increased body weight is due to increased fat mass[15].

On the other hand, after streptozotocin injection and development of T2DM there was significant increase in food intake and significant decrease in body weight in diabetic group in comparison with control group. The increased food intake is as a result of decreased activity of the leptin receptor, impaired glucose utilization and increase energy demand. While, the decreased body weight is due to increased catabolic reactions with subsequent muscle wasting which may be the major cause for weight loss in diabetic rats [16].Metformin reduces food intake in this study could be explained by attenuating neuropeptide–Y (NPY)[17]. Additionally, metformin increases enterocyte glucose utilization in an anaerobic manner with local lactate production. This gut lactate production may drive some of the gastrointestinal symptoms associated with metformin including GI discomfort which in turn decreases appetite[18]. Also, metformin stimulates glucagon like peptide-1(GLP-1) which inhibits food intake [19].

On the other hand, metformin induces weight loss through activating lipolysis by inhibiting adipogenesis. It also inhibits carbohydrate absorption and bile salt uptake through stimulation of GLP-1 which inhibits energy production[19].

Hyperglycemia in diabetic group could be explained by decrease glucose uptake in peripheral tissues. Also, these increase hepatic gluconeogenesis through increasing uptake of gluconeogenic precursors and increasing the activity of gluconeogenic enzymes[20]. Furthermore, defected insulin action leads to increase lipolysis and FFA oxidation which produce a gluconeogenic precursor called glycerol [21].

Metformin decreases blood glucose level in the present study through several mechanisms. These include inhibition of intestinal glucose absorption, suppression hepatic glucose production, reduction in hepatic glucose output, facilitation of glucose uptake by tissues and improving insulin sensitivity [22].

Metformin increases glucose uptake in skeletal muscle via increased translocation of GLUT4 (glucose transporter type 4) transporters to the plasma membrane[23]. Metformin decreases blood glucose through increasing the activity of the insulin receptor and its substrate which increases the uptake of glucose in the liver cell [24].

This hyperglycemia causes oxidative stress that decreases insulin biosynthesis and secretion. Also, there are apoptotic β-cell death , impaired K_{ATP} channel, mitochondrial dysfunction and reduction in expression and/or activities of several insulin gene transcription factors such pancreatic and duodenal homobox-1 (PDX-1) [25].This explain decreased insulin in diabetic group

Metformin increased serum insulin level in this study could be explained by enhancing the expression and function of the glucose transporter-4 in cells and so its ability to stimulate insulin secretion[26]. It also, stimulates GLP-1 (glucagon-like-peptide-1) release, thereby enhancing insulin secretion[27]. Moreover, it increases aquaporin 7 (AQP7) expression with subsequent glycerol influx into β-cell, so increasing insulin secretion [28].

The pathophysiology underlying diabetic induced cardiac damage with increase serum levels of cardiac injury markers which include total creatine kinase and creatine kinase MB and LDH is complex and is related to multiple factors. These include altered myocardial energy substrate, glucotoxicity, lipotoxicity, increased oxidative/nitrosative stress, activated renin-angiotensin and adrenergic system, endothelial dysfunction, insulin resistance, maladaptive immune response, advanced glycation end products (AGEs), mitochondrial dysfunction, abnormal calcium handling, inflammation and cell death[29]**.** These may explain the data of the present study.

In diabetic heart, there is metabolic inflexibility in which heart shifts from glucose to fatty acid oxidation to provide energy. This increases ATP expenditure and inhibits ATP shuttling from mitochondria to the cytosol. It also increases the expression of mitochondrial uncoupling protein (UCP) 3 which disturbs the mitochondrial proton gradient and cause inefficient ATP production. These all interfere with excitation–contraction coupling and mitochondrial Ca^{2+} uptake and finally, these changes produce oxidative stress and mitochondrial dysfunction[30]**.** Additionally, excessive fatty acid (FA) oxidation leads to accumulation of toxic lipids such as diacylglycerol (DAG) and ceramids in the cytosol of cardiomyocytes which in turn activate protein kinase C (PKC) isoforms that reduces insulin metabolic signaling and leads to oxidative stress [31].

On the other hand, insulin resistance (IR) and hyperglycemia cause inappropriate activation of renin – angiotensin – aldosterone system (RAAS) which in turn enhances the adaptive proinflammatory immune response and inflammation[32]**.** Additionally, persistent hyperglycemia causes toxic advanced glycated end products (AGEs) formation which impair the sarcoplasmic reticulum (SR) Ca^{2+} reuptake in cardiomyocytes[33] and enhance extracellular matrix (ECM) degeneration with subsequent myocardial fibrosis and stiffness[34].

On the other hand, in metformin treated group, the data of the current study showed insignificant decrease in serum levels of cardiac injury markers which include total creatine kinase, creatine kinase MB (cK-MB) and LDH than diabetic group. Metformin ameliorates glucose and lipid induced cardiac injury, ROS production, cardiomyocyte apoptosis and fibrosis. Metformin can activate prokineticin 2 (PK2) which is a protein that protects against oxidative stress in cardiomyocytes and reverses apoptosis. Also, PK2 inhibits hyperglycemia-induced cardiac contractile dysfunction and enhances the strength of contraction. In addition, PK2 can protect cardiomyocytes from apoptosis. Furthermore, PK2 enhances epicardium-derived progenitor cells involved in tissue repair/regeneration in heart diseases. PK2 induces the growth of myocardium, myocardial survival and angiogenesis [35].

Additionally, metformin promots autophagy[36]**..** It also counters mitochondrial dysfunction by inhibition excessive mitochondrial fission which lead to myocardial injury. It attenuates cardiac mitochondrial dysfunction by decreesing mitochondrial ROS production and mitochondrial swelling [37].

The data of the present work revealed markers of oxidative stress in diabetic group in the form of significant increase in serum level of MDA and significant decrease in serum level of total antioxidant than control group. These were resulted from either abnormal glucose metabolism or lipid metabolism. In hypergly-cemic conditions, there is excessive production of superoxide anion radical (O_2^-) which suppresses the body antioxidant systems and so induce oxidative stress and damage nuclear DNA as well as other biomolecules [38].

DNA damage leads to activation of a DNA repair enzyme which inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This results in increased levels of glyceraldehyde-3-Phosphate (GAP)**.** Additionally, accumulated glyceraldehydes-3-Phosphate leads to glycerol-3-Phosphate which in turn combines with fatty acids to drive the de novo synthesis of diaclyglycerol (DAG). The increased cellular level of diaclyglycerol (DAG) up regulates protein kinase C (PKC) pathway which in turn stimulates reactive oxygen species (ROS) generating enzymes such as NADPH-oxid-

ases and lipooxygenses with subsequent exacerbation of cellular oxidative environment [39].

On the other hand, in hyperglycemia, the generation of fructose aids in the formation of the precursors of advanced glycation product (AGEs). AGEs generate oxidative stress by binding with its receptor RAGE thereby causing oxidative damage through nonenzymatic glycation [40]. Similarly, hyperglycaemia and hyperlipidemia lead to increased production of NADH and $FADH_2$ which are used by the mitochondrial electron transport chain (ETC) to generate ATP. This NADH overproduction causes higher proton gradient production in mitochondria, with the surplus electrons being transferred to O_2 to produce $O_2^{\cdot-}$ [41].
Metformin decrease oxidative stress markers in present study by enhancing glucose uptake into liver and skeletal muscle. Metformin provides an intracellular antioxidant properties through inhibition of protein kinase C activity[42]. It decreases mitochondrial ROS production by protecting mitochondria from tissue growth factor - β1 (TGF-β1) induced damage and upregulates the mitochondrial antioxidant system [43].

Conclusion

Metformin is an oral antidiabetic drug that reduces blood glucose, increases insulin, improves the lipid profile and has beneficial effects on the heart. It also alters the oxidative stress and increases antioxidant capacities. These effects are evidenced by improving the cardiac injury markers and cardiac histopathological picture.

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