

# Therapeutic potential of oxymatrine in impeding the cardiomyopathy in the STZ- nicotinamide induced diabetes through SIRT1/Nrf2 signaling activation

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## Research Article

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# Abstract

## Aims

Diabetes mellitus increases the risk of heart failure independently of underlying hypertension and ischemic heart disease, leads to the cardiomyopathy. Molecular mechanism underlying these pathological changes in the diabetic cardiomyopathy (DCM) are most likely to multifactorial, but clearer pathogenesis is partially understood. Literature showed that insulin resistance was associated with the dysfunction of SIRT1, TGF- $\beta$ 1 protein expression and pro-apoptotic pathways. In the current research, we aimed to investigate the ameliorative effect of oxymatrine (OMT) against streptozotocin-nicotinamide (STZ-NA) induced DCM in the experimental animals.

## Method

Male wistar rats (120–150 g) were pre-treated with the NA (110 mg/kg, i.p) followed by administration of STZ (60 mg/kg, i.p) after 15 min. After observed the onset of cardiomyopathy evaluated by increased diastolic dysfunction followed by systolic, two weeks later of STZ-NA administration, animals were divided in to various treatment groups. Diabetic animals were treated with pioglitazone (10mg/kg, p.o) and OMT(25, 100, 150 mg/kg, i.p) for 3 weeks. Various biochemical parameters were checked after completion of the experimental protocol.

## Key findings:

Diabetic animals showed hyperglycemia, impaired glucose tolerance and lipid profile. In addition, increased blood pressure, serum LDH, CK-MB levels, and abnormal hemodynamic. Apart from this, pro-inflammatory cytokines, apoptotic markers, TGF- $\beta$ 1 activity were increased and SIRT1 activity was decreased in the diabetic animals. While, treatment with the OMT, restored all these abnormalities. Additionally, OMT treatment dose dependently restored the anti-oxidants, pro-inflammatory, and apoptotic marker. On the basis of these observations, we concluded that OMT can protect diabetic rats from insulin resistance through the regulation of SIRT1/Nrf2, TGF- $\beta$ 1 and pro-apoptotic pathways.

## Introduction

Diabetic patients share a 74% more risk of developing heart failure (HF) than age-matched non-diabetic individuals, regardless of other comorbidities, making cardiovascular illnesses the leading cause of mortality in this population. Research of Rubler et al., (1972) reported the autopsy data of diabetic patient with left ventricular (LV) dilation in the absence of any sign of heart failure (Rubler et al., 1972). Later reports confirmed the concept of diabetic cardiomyopathy (DCM). Studies indicate that people with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) share 14.5% and 35.0% outbreak of DCM (Zaveri et al., 2020). The pathophysiological underlying the development of DCM are multifactorial,

indeed it is incompletely understood. Furthermore, research indicate that hyperglycemia mediated oxidative stress, lipotoxicity, myocardial inflammation, apoptotic cascade, metabolic dysregulation, vascular changes, remodelling and fibrosis might be contributed to the development of DCM.

Pathophysiology of DCM is not limited to oxidative stress, apoptosis has been also linked with the pathogenesis of DCM. However, oxidative stress induced by metabolic malfunctioning is one of the key reasons in the development of DCM. However, overgeneration of reactive oxygen species (ROS) due to mitochondrial malfunction further leads to activation the apoptotic pathway and increase the production of inflammatory cytokines in the myocardium which exacerbates myocardial fibrosis, contractile dysfunction as well as left ventricular hypertrophy, cardinal sign of DCM (Kaludercic & Di Lisa, 2020). Moreover, reports suggested that hyperglycemia contributes modulating the AMPK(Lin et al., 2017), SIRT1 (Karbasforooshan & Karimi, 2017), Nrf2(Li et al., 2019) and TGF- $\beta$ 1 (Lorenzo-Almorós et al., 2017) signalling pathways. Meanwhile, studies indicated that, activation of SIRT1 leads to activation of AMPK and Nrf2 cascade expression which shows beneficial anti-oxidant, anti-inflammatory, metabolic regulation and anti-apoptotic effect (Ma et al., 2020). Moreover, the integrity of the cardiac cells depends on functional autophagy, which is a dynamic process that is carefully regulated by cellular metabolic balance. Literature indicated that increased expression of TGF- $\beta$ 1/smad signaling in hyperglycemic condition leads to increased the collagen and extracellular matrix (ECM) deposition in the cardiomyocytes results in myocardial fibrosis and left ventricular hypertrophy(Meng et al., 2019). Thus, drugs that act on these targeted molecular cascades can be therapeutic strategy for treating cardiomyopathy in the diabetic patient.

Natural plant products are interestingly accepted as health promoting agent, oxymatrine (OMT) is alkaloid extracted from the traditional Chinese herb *Sophora flavescens*, has various therapeutic effects like anti-inflammatory, anti-hyperglycemic, anti-viral, anti-oxidative, anti or pro-apoptotic, anti-fibrotic and attenuating HF property. Additionally, experimental evidence revealed that OMT also possessed anti-hyperglycemic activity in the diabetic animal models(P. Zhao et al., 2015). Previous studies have shown that OMT can improve myocardial infarction by attenuating myocardial hypertrophy, left ventricular dysfunction and heart failure (HF) (Shen, Yang, Xiao, Peng, & Liu, 2011). Research indicate that the anti-inflammatory response of OMT is exert through down regulation of NF- $\kappa$ B pathways, toll like receptor (TLR) signaling pathway and reduce the pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, IL-13, TNF- $\alpha$ , transforming growth factor (TGF- $\beta$ 1) and CRP. Study of Lu et al., (2016) indicated that, OMT could inhibit the ROS production by upregulating Nrf2/HO-1 signaling pathway and inhibit apoptosis by downregulation of the TLR/PI3K/Akt/ GSK3- $\beta$  signaling pathway(Lu, Xiang, & Xia, 2016). Previous studies have shown that OMT can improve myocardial infarction by attenuating myocardial hypertrophy, left ventricular dysfunction and heart failure (HF) (Shen et al., 2011). Furthermore, recent evidence revealed that OMT can inhibit the proliferation of cardiac fibrosis induced by aldosterone (L. Zhao et al., 2018). Beside the numerous pharmacological properties, the protective effect of OMT on DCM has not been reported to date. However, recent studies indicate that OMT showed the potential effect in non-alcoholic steatosis through AMPK/SIRT1 pathway activation (Xu et al., 2020). Therefore, the current research was

developed to test the protective effect of OMT and understand its molecular mechanism of DCM using streptozotocin and nicotinamide induced T2DM experimental animal model.

## Materials And Methods

### 2.1 Experimental animals

The male wistar rats, (14–16 weeks, weighing 200–220 g), were used in this experimental protocol and kept under a standard environment ( $22 \pm 3$  °C), with a ( $55 \pm 6\%$ ) humidity and (12:12 hrs light/dark cycle). Wistar rats were purchased from animal house of ISF college of pharmacy, Moga, Punjab, India (Reg no.816/PO/ReBiBt/S/04/CPCSEA). Tap water and standard pellet diet under sanitary conditions were fed to the animals. All the experimental animal protocols used in this research work were performed accordance with the regulations specified by the Institutional Animal Ethics Committee (IAEC), ISF college of pharmacy, Moga, Punjab. The experimental protocol were strictly followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India.

### 2.2 Induction of T2DM in animals

In this experimental animal model, overnight fasted rats would receive single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg, i.p) dissolved in 0.1 M Sodium citrate, pH 4.5 (STZ; HI Media, Mumbai, India) which would followed by nicotinamide (110 mg/kg, i.p) administration after 15 minutes (Mishra, Verma, & Vijayakumar, 2013). After ~ 72 h of STZ administration, blood samples would be collected from the tail veins of the animals, and the blood glucose levels  $> 250$  mg/dL with disturbed glucose tolerance would considered T2DM animals. Two weeks after the STZ-nicotinamide administration, animals would and only divided in to the six groups ( $n = 8$ ) as following: normal group: received saline (1 ml/kg/day, i.p.), diabetic control, diabetic animals treated with pioglitazone (10 mg/kg, p.o ) and multiple doses of OMT (25, 100, 125 mg/kg, i.p) for 21 days. After completion of the treatment schedule of 21 days, animals were used for hemodynamic parameters using Langendorff's apparatus and blood samples were collected for further biochemical markers. Hearts were removed and gently washed with ice-cold saline, and homogenized in cold phosphate buffer (PBS, 0.1 M, pH = 7.4) for different biochemical investigation.

### 2.3 Determination of % body weight change

The % body weight change in each group of animals were monitored after completion of the study to ensure the effect of various treatment in body weight. Body weight of each animal was recorded on the first and last day of experiment. The percentage change in body weight was calculated as-

$\% \text{ body weight change} = (\text{Final body weight} - \text{Initial body weight}) / \text{Final body weight} \times 100.$

### 2.4 Determination of blood glucose level

Fasting blood glucose levels in each group of animals were monitored weekly using Dr. Morepen Glucone glucometer. To check the FBG, food were withdraw from the cages and rats were fasted overnight

before the test. Glucose levels were checked during 9:30 am to 11:30 am.

## 2.5 Oral glucose tolerance test

Oral glucose tolerance test (OGTT) is a typical clinical test for determining whether a patient has T2DM and glucose intolerance (Muniyappa, Lee, Chen, & Quon, 2008). A night before the test food was removed from animal cage. The animals were administered with glucose (2 g/kg p. o, dissolved in saline). Glucose levels were measured using glucometer at 0, 30, 60, and 120 min after the bolus.

## 2.6 Determination of lipid profile

Serum total triglyceride, cholesterol, LDL and HDL levels were measured by an enzymatic colorimetric method using commercial assay kits (Accurex and Coral). Briefly, into the EDTA coated tubes blood sample was collected, and plasma was separated by the centrifugation at (10,000 rpm for 15 min, at 4°C) and the supernatant was collected for the estimation of total triglycerides, cholesterol, LDL and HDL. Briefly, 0.1 ml of plasma supernatant, 1 ml of prepared working reagent (prepared according to the Accurex and Coral diagnostic kits instruction). The absorbance was read at the 500–510 nm against blank without sample.

**Calculation: TG (mg/dL):  $\text{conc. (mg\%)} = \text{abs of sample} / \text{abs of standard} \times 200$**

**TC (mg/dL):  $\text{conc. (mg\%)} = \text{abs of sample} / \text{abs of standard} \times 200$**

**HDL (mg/dL):  $\text{abs Test} / \text{abs Standard} \times 25 \times 2$  (2 is the dilution factor due to the deproteinization step).**

**LDL (mg/dL):  $\text{TC} - \text{HDL} - (\text{TG} / 5)$**

## 2.7 Determination of Non-invasive blood pressure (NIBP)

Systolic and diastolic blood pressure were measured after completion of study in each group using NIBP 200A small animal tail non-invasive blood pressure system (BIOPAC systems, Inc).

## 2.8 Determination of serum CK-MB and LDH

Blood serums were collected using retro-orbital method after completion of dosing in each group. CK-MB and LDH is a cardiac biomarker, determined in blood serum in each group using commercial kit purchased from (Coral clinical systems) followed by instructions given in kit respectively.

## 2.9 Determination of haemodynamic parameters of heart

After completion of study the animals were sacrifice and immediately heart was isolated and mounted on digital Langendroff apparatus (RADNOTI, California, USA) and perfused with Krebs-henseleit solution, gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (Carbogen gas) pH 7.4 maintained at 37°C, further a latex balloon filled with 95% methanol was inserted lightly into left ventricle (LV) over the left atrium appendage through the mitral valve for the assessment of various cardiac haemodynamic parameters such as  $dp/dt_{\text{max}}$ ,  $dp/dt_{\text{min}}$ , LVEDP were measured using a pressure transducer (Biopac-MP100) system. After obtaining the

readings, the heart was detached from the apparatus and dried. Heart weight calculation was done, and then it was frozen at -20°C for 6–7 hours.

## 2.10 Determination of HW/BW ratio % change

After completion of hemodynamic parameters HW/BW ratio % change was determined in each group respectively using the following formula.

**Formula: Heart weight (g)/ Body weight(g)× 100**

### 2.11 Estimation of biochemical parameters

After completion protocol rats were killed for biochemical, inflammatory, and histopathological analysis, respectively. For histological analysis, the heart was separated by being placed on ice and then perfused with 10% formaldehyde. Additionally, the hearts that were extracted for the assessment of biochemical markers were washed with an ice-cold, isotonic saline solution at 37°C and homogenised with an equivalent amount of ice-cold, 0.1 M phosphate buffer saline (pH 7.4). The homogenate was next centrifuged for 15 minutes at 4°C (10,000 rpm) to separate out aliquots of the homogenate for use in estimating biochemical parameters. According to the Wills 1965 approach, the end product of lipid peroxidation, known as lipid peroxidation (LPO), was measured in cardiac homogenate (Wills, 1965). Moreover, activity of Catalase (CAT) was assayed according to the procedure given by Orr and Sohal, in which the breakdown of H<sub>2</sub>O<sub>2</sub> is measured at 240 nm (Aebi, 1974; Orr & Sohal, 1992). Further reduced glutathione (GSH) was estimated with the help of Ellman's reagent and the process mentioned in previous studies.

### 2.12 Determination of Caspase3 activity and pro-inflammatory cytokines expression

Determination of caspase3 activity in cardiac tissue homogenate was performed by sandwich-ELISA principle of Elabscience CASP3 (caspase3) ELISA kit. And The quantitative estimation of pro-inflammatory cytokines (TNF-α, IL-1β) were done by ELISA kits (Fine Test, Wuhan, China) according to method described by.

### 2.13 Determination of Nrf2 activity

Determination of Nrf2 activity in heart tissue homogenate was done by sandwich-ELISA principle using Nrf2 ELISA kit (Elabscience, USA). When NFE2L2 is introduced, it binds to antibodies that have been coated on the wells in the tissue homogenate. After that biotinylated Rat NFE2L2 antibody is added and binds to NFE2L2 in the sample. The biotinylated NFE2L2 antibody then binds to streptavidin-HRP, which is then added. During the washing phase after incubation, unbound streptavidin-HRP is removed. The amount of Rat NFE2L2 is then correlated with the development of colour in the substrate solution. By adding an acidic stop solution, the process was stopped, and the colour changed to yellow. A wavelength of 450 nm + 2 nm was used to spectrophotometrically determine the optical density (OD).

## 2.14 Determination of SIRT-1 and TGF- $\beta$ activity

These ELISA kits followed the sandwich-ELISA principle purchased from (Elabscience, USA). The micro ELISA plate provided in kits were pre-coated with an antibody specific to rat TGF- $\beta$  1, SIRT1, respectively. Samples are added into the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated antibody for Rat, TGF- $\beta$ , SIRT1 and Avidin-HRP conjugate were added gradually to the well of each micro plate and incubated. In the washing step free components were washed away. Only the wells in the kits that contained the appropriate amounts of Rat TGF-, SIRT1, biotinylated detection antibody, and Avidin-HRP conjugate were blue in colour. The addition of stop solution put a halt to the enzyme-substrate reaction, and the colour changed to yellow. The optical density (OD) then determined at a wavelength of  $450 \text{ nm} \pm 2 \text{ nm}$ .

## 2.15 Histopathological studies

After killing the animals, heart were isolated and fixed in 10% formalin, embedded in paraffin and then sectioned ( $5 \mu\text{m}$ ). Hematoxylin and eosin was used to stain the sectioned tissues. Cells were detected according to the shape of nuclei at 40X magnification. Photomicrographs and a motic fluorescent microscope were used to assess any histological alterations.

## Statistical analysis

Graph Pad Prism 5.0 software (Graph Pad Software, Inc., USA) were used for the statistical analyses. The values were expressed as means  $\pm$  standard errors of the mean (SEMs) ( $n = 8$ ). The differences between several groups were statistically analysed by using one-way and two-way analyses of variance (ANOVA), followed by post-hoc tests. Values of  $P < 0.05$  were deemed to be statistically significant.

# Results

## 5.1. Effect of OMT on% body weight change

After completion of experimental protocol our result indicated that the %body weight of the diabetic animals was significantly decreased as compared to the normal group ( $*p < 0.05$ ) as shown in the Fig. 1. However, the %body weight of animals treated with pioglitazone was significantly increased/ restored as compared to the diabetic group ( $\#p < 0.05$ ). OMT treatment at the low dose (25 mg/kg, i.p) did not change the %body weight in diabetic rats, while at the dose of (100, 150 mg/kg, i.p) remarkably restored the %body weight in diabetes ( $\#p < 0.05$ ). Interestingly, OMT treatment at the higher dose showed similar %body weight change as with pioglitazone treatment in diabetic animals.

## 5.2 Effect of OMT on blood glucose level

After inducing T2DM in animals with STZ and NA, the fasting blood glucose level of the rats were measured at the end of the study as shown in the Fig. 2. Compared with the normal group ( $110 \pm 10$

mg/dL) the FBG of T2DM rats were increased significantly ( $490 \pm 10$  mg/dL)(\* $p < 0.05$ ). Diabetic animals administered with OMT and it was observed that OMT could reduce the FBG in a dose dependent manner and our findings showed that at higher dose (150 mg/kg, i.p) demonstrated similar level of restoration of FBG level as compared to the standard group. Thus, our results suggested that OMT effective for lowering blood glucose level in the diabetic/T2DM animals.

## 5.3 Effect of OMT on OGTT

Glucose tolerance was checked using the oral glucose tolerance test. Compared to the normal group diabetic animals showed a significant elevation (\* $p < 0.05$ ) of FBG at the time points 0, 30, 45, 60 and 120 minutes. Our result indicated that diabetic rats were showed significant glucose impairment. While treatment with different doses of OMT (100, 150 mg/kg, i.p) for 21 days, the blood glucose levels at time point 0, 30, 45, 60, 120 were reduced significantly. Furthermore, higher dose of OMT exhibited similar effect as compared to the rats treated with pioglitazone.

## 5.4 Effect of OMT on Serum lipid profile

At the end of the experimental protocol lipid profile in the STZ-NA induced T2DM animals were significantly impaired (increase in TG, TC, LDL level and decrease in HDL level) as compared to the normal group (\* $p < 0.05$ ) as shown in the Fig. 4. However, treatment with pioglitazone significantly restored the lipid profile when compared with the diabetic group ( $\#p < 0.05$ ). Moreover, our result indicated that treatment with OMT (100, 150 mg/kg, i.p) remarkably improved the lipid profile (decrease in TG, TC, LDL level and increase HDL level) in STZ-NA induced diabetic rats. Moreover, treatment with higher dose of OMT exhibited similar effect in restoration of lipid profile as compared with the pioglitazone treated group. Thus, our result indicated that OMT treatment can reduce the risk of hyperlipidemia and associated cardiovascular risk factors in T2DM animals.

## 5.5 Effect of OMT on systolic and diastolic blood pressure

After completion of the experimental protocol our findings suggested that the systolic and diastolic blood pressure of STZ-NA induced diabetic rats increased significantly as compared to the normal group (\* $p < 0.05$ ) as shown in Table 1. However, treatment with pioglitazone (10 mg/kg, p.o) shows significant decrease in the systolic and diastolic blood pressure as compared with the diabetic group ( $\#p < 0.05$ ). OMT treatment at the dose of (100, 150 mg/kg, i.p) resulted in significant reduction in the systolic and diastolic blood pressure as compared to the diabetic group. Furthermore, our result demonstrated that treatment with higher dose of OMT produce similar effect when compared with the pioglitazone treated animals.

## 5.6 Effect of OMT on heart weight/body weight % change (HW/BW)

To explore the ameliorative effect of OMT in STZ-NA induced diabetic cardiac hypertrophy, we measured the HW/BW% change were checked after completion of the experimental protocol. Compared to the



normal group diabetic animals exhibited significant increase in HW/BW% change ( $*p < 0.05$ ) as shown in the Fig. 5, suggesting cardiac hypertrophy. Our result indicated that treatment with OMT low dose (25 mg/kg, i.p) did not show any positive effect on the HW/BW% change. While, OMT treatment at the dose of (100, 150 mg/kg, i.p) significantly decline the HW/BW% change as compared to the diabetic animals ( $\#p < 0.05$ ). However, higher dose of OMT showed more significant effect in HW/BW% restoration as compared to the D + PIO group.

## 5.7 Effect of OMT on hemodynamic parameters of heart

The cardio-protective effect of OMT further confirmed by observed its effect on hemodynamic parameters of heart. Our result shows that upon STZ-NA administration there were a significant rise in LVEDP and decline in  $dp/dt_{max}$  and  $dp/dt_{min}$  in diabetic animals as compared with the normal group ( $*p < 0.05$ ) as shown in Fig. 6, Indicating contractile dysfunction of diabetic heart. Further, treatment with pioglitazone (10 mg/kg, p.o), significantly decrease the LVEDP and increase the  $dp/dt_{max}$  and  $dp/dt_{min}$  as compared with the diabetic group ( $\#p < 0.05$ ). However, significant improvement in hemodynamic parameters (LVEDP,  $dp/dt_{max}$  and  $dp/dt_{min}$ ) were observed in OMT (100, 150 mg/kg, i.p) administered group as compared with the diabetic animals ( $\#p < 0.05$ ). Interestingly, our result indicated that OMT treatment at higher dose showed significant recovery in hemodynamic parameters as compared to the standard group. Thus, OMT administration can ameliorate the contractile dysfunction in T2DM animals.

## 5.8 Effect of OMT on serum cardiac marker enzymes

Increased circulation of CK-MB and LDH in blood serum indicates myocardial damage, according to our result at the end of the study there were significant increase in CK-MB and LDH in blood serum in diabetic rats as compared with normal group ( $*p < 0.05$ ) as shown in Fig. 7. However, treatment with pioglitazone (10 mg/kg, p.o) significantly decreased the serum LDH and CK-MB level as compared with the diabetic group ( $\#p < 0.05$ ). Further, low dose of OMT could not reduce these factors in diabetics. While, administration of OMT at the dose of (100, 150 mg/kg, i.p) significantly reduced the serum LDH and CK-MB level when compared with the diabetic animals ( $\#p < 0.05$ ). Whereas, our findings suggested that OMT treatment at high dose restored the serum LDH and CK-MB level significantly when compared to the pioglitazone treated group.

## 5.9 Effect of OMT on oxidative stress biomarkers

Increased blood glucose level and lipid profile are linked with excessive generation of ROS; so, in this study we evaluate the effect of OMT on oxidative stress biomarkers in diabetic heart. Our findings indicated that in the diabetic rat heart there were a significant reduction in GSH, CAT activity and rise in MDA level compared with normal group ( $*p < 0.05$ ) as shown in the Fig. 8. Whereas, treatment with pioglitazone (10 mg/kg, p.o) significantly increased the GSH, catalase level and significantly decline the MDA level as compared with the diabetic animals ( $\#p < 0.05$ ). Conversely, OMT treatment at the dose of (100, 150 mg/kg, i.p) for 3 weeks significantly increased the GSH, catalase activity and decrease the MDA

level as compared with to the diabetic group. However, significant improvement in oxidative biomarkers were seen in D + OMT3 group as compared to the D + PIO group.

## 5.10 Effect of OMT on inflammatory cytokine

When compared to normal group and diabetic group, the level of TNF- $\alpha$  and IL-1 $\beta$  were increased significantly in diabetic heart (\* $p < 0.05$ ), Fig. 9. However, administration of pioglitazone (10 mg/kg, p.o) significantly reduced the level of TNF- $\alpha$  and IL-1 $\beta$  as compared with the diabetic group ( $\#p < 0.05$ ). Further, no amelioration of pro-inflammatory cytokines were observed in D + OMT1 group. While, treatment with OMT at the dose of (100, 150 mg/kg, i.p) remarkably reduced the TNF- $\alpha$  and IL-1 $\beta$  when compared to the diabetic animals ( $\#p < 0.05$ ). Whereas, OMT at higher dose exhibited significant effect in reducing the pro-inflammatory cytokines level when compared to the standard group.

### 5.11 Effect of OMT on caspase3 activity

To assess the impact of OMT in ameliorating apoptosis in diabetic rat heart, we examined the expression level of caspase3. The caspase3 activity was observed significant increase in STZ-NA treated rats when compared to the normal group (\* $p < 0.05$ ) as represented in Fig. 10. However, pioglitazone treatment at (10 mg/kg, p.o) dose shows significant decline in caspase3 activity as compared to the diabetic group ( $\#p < 0.05$ ). Moreover, lower dose of OMT does not demonstrate any significant effect in caspase3 activity. Whereas, OMT treatment at (100, 150 mg/kg, i.p) for 3 weeks significantly reduced the caspase3 expression rather compared to the diabetic group ( $\#p < 0.05$ ). Furthermore, compared to the standard group treatment with OMT higher dose showed significant effect in caspase3 activity.

### 5.12 Effect of OMT on Nrf2 expression

It has been reported that increased expression of Nrf2 leads to regulates the levels of antioxidant proteins to protect cells against oxidative damage. Nrf2 expression were analyzed after completion of the experimental schedule, diabetic animals exhibited a significant decrease in Nrf2 expression when compared with the normal group (\* $p < 0.05$ ) as represented in Fig. 5.10. Further, significant increase in Nrf2 expression was observed upon treatment with pioglitazone (10 mg/kg, p.o) as compared to the diabetic group ( $\#p < 0.05$ ). OMT treatment at (100, 150 mg/kg, i.p) dose showed significant rise in Nrf2 expression in diabetics. Interestingly, significant improvement in Nrf2 expression was observed in the high dose of oxymatrine group when compared to the standard group.

### 5.13 Effect of OMT on SIRT1 and TGF- $\beta$ 1 expression

Previous study reported that increased expression of SIRT1 leads to metabolic regulation, decrease oxidative stress, apoptosis and inflammation. Whereas, increased expression of TGF- $\beta$ 1 reported to increase myocardial fibrosis. At the end of our experimental protocol our findings suggested that there were significant decrease in SIRT1 expression and increase in TGF- $\beta$ 1 expression in diabetic heart when compared with the normal group (\* $p < 0.05$ ) as depicts in the Fig. 11a and b. Further, treatment with

pioglitazone (10 mg/kg, p.o) showed a significant increase in SIRT1 and decrease in TGF- $\beta$ 1 expression as compared with the diabetic group ( $^{\#}p < 0.05$ ). Moreover, no significant effects were seen after administration of lower dose of OMT. Whereas, treatment with OMT at the dose of (100, 150 mg/kg, i.p) for 21 days represent significant up-regulation in SIRT1 expression and down-regulation in TGF- $\beta$ 1 expression when compared to the STZ-NA treated animals. However, significant improvement in SIRT1 and TGF- $\beta$ 1 expression were demonstrated in high dose OMT group as compared to the D + PIO group.

#### **5.14 Effect of OMT on cardiac fibrosis histopathological studies**

The H&E-stained section of myocardium in diabetic group Fig. 12 showed collagen deposition and interstitial fibrosis in heart tissue as compared to the normal myocardium fibers. However, treatment with pioglitazone (10 mg/kg, p.o), OMT (100 mg/kg, i.p) and OMT (150 mg/kg, i.p) showed restoration the normal architecture of the myocardial fibers as compared to the diabetic group (Fig. 12c, e and f). Further, these favourable effects were more pronounced in the group treated with the pioglitazone (10 mg/kg, p.o) and OMT (150 mg/kg, i.p) relative to rats treated with OMT (25 mg/kg, i.p) (figure.12d) and OMT (100 mg/kg, i.p). Moreover, OMT high dose group showed more significant effect rather compared to OMT intermediate dose (100 mg/kg, i.p) and OMT low dose (25 mg/kg, i.p).

#### **5.15 Effect of OMT on myocardial histopathological damage**

The H&E stained section changes in rat myocardium were graded and scored into four grades, i.e., absent (0), mild changes (#), moderate changes (##), severe changes (###) as mentioned in (Table. 2). The heart of normal group demonstrated normal morphology with cell borders, normal cytoplasm, homogeneous oval nuclei without any inflammation and necrosis in normal group (figure.13a). However, in the diabetic group (figure. 12b), intercellular border was obscure with a loss of nuclear integrity existed in cardiomyocyte. The changes also showed necrosis, pyknotic cells, infiltration of inflammatory cells and disrupted cytoplasm. Moreover, histopathology of rats' heart treated with pioglitazone (10 mg/kg, i.p), OMT (100 mg/kg, i.p) and OMT (150 mg/kg, i.p) revealed declined infiltration of inflammatory cells, necrosis and restored the normal morphology of cardiac cells with normal nuclei and cytoplasm (figure. 13c, e and f). Further, these protective effects were more obvious in the group treated with the pioglitazone (10 mg/kg, p.o) and oxymatrine (150 mg/kg, i.p) relative to rats treated with OMT (25 mg/kg, i.p) (figure.12d) and OMT (100 mg/kg, i.p). However, more pronounced effect was seen in OMT high dose (150 mg/kg, i.p) treated group than OMT intermediate dose (100 mg/kg, i.p) and OMT low dose (25 mg/kg, i.p) treated group.

## **Discussion**

In the current research, we have explained that oxymatrine effectively restored the anti-oxidants level, reduced the inflammation and attenuate the DCM through restoration of SIRT1/Nrf2 signaling pathway via normalizing disturbed autophagy and decreasing apoptosis in the diabetic rats. Diabetes has been linked to hyperglycemia, hyperlipidemia and insulin resistance that leads to increase oxidative stress,

inflammation, apoptosis, fibrosis, cardiomyocyte hypertrophy, systolic and diastolic dysfunction and consequently diabetic cardiomyopathy (DCM). Our findings showed that reduction of cardiac nuclear translocation of Nrf2 and SIRT1 were accompanied by increased apoptosis, fibrosis (TGF- $\beta$ 1) and consequently cardiac malfunction in the STZ-nicotinamide induced diabetic rats, which could be reversed by the oxymatrine.

In the present study STZ-NA induced diabetic animals was used to examine the defensive effect/mechanism of oxymatrine against cardiomyopathy. Excessive generation of oxidative agents, which results in onward damage to the pancreas and other tissues, is associated with long-term hyperglycemia. Various research showed that  $\beta$ -cells are extremely vulnerable to oxidative damage that in turn can aggravate hyperglycemia (Wang & Wang, 2017). Our results showed that oxymatrine ameliorates against diabetes-induced cardiomyopathy by modulating the cardio-metabolic risk factors, oxidative stress, inflammation, cell death and fibrosis through activating the SIRT1/Nrf2 signaling cascade.

In our study we found that administration of STZ- nicotinamide was associated with reduction in the body weight of the rats due to degradation of proteins in muscular tissue results from insulin deficiency (Sundaram, Naresh, Shanthi, & Sachdanandam, 2013), it was confirmed by calculating the % body weight change in different groups. Furthermore, our findings indicated that administration of oxymatrine at high dose for 21 days significantly increase the body weight as compared to the diabetic animals. Insulin producing and Insulin sensitivity medications are widely used in the treatment of patient with T2DM. Interestingly, our results showed that oxymatrine treatment significantly reduced the pre-prandial and post-prandial blood glucose level. We presume that there are several mechanisms of anti-hyperglycemic effect of oxymatrine, firstly: it may increase the insulin sensitivity in target tissue thereby increase glucose uptake that produces anti- hyperglycemic effect. Secondly: it increases the regulation of SIRT-1 that is interconnected with AMPK. It has been shown that, SIRT1 deacetylates the AMPK kinase LKB1, leading to increased phosphorylation and activation of AMPK (Lan, Cacicedo, Ruderman, & Ido, 2008). Activation of AMPK is associated with increases in glucose uptake through an insulin-independent mechanism.

Findings of OGTT reflects the body's efficiency in disposing of glucose and is a widely used test for an indirect assessment of insulin resistance in animals. In the current investigation, treatment with oxymatrine high dose significantly decrease the FBG and increase body's glucose tolerance capacity which indicates oxymatrine ameliorates the insulin resistance and metabolic dysregulation. In diabetes impaired lipid profile is associated with lipid accumulation and intra-myocardial lipotoxicity which leads to increase the risk of systolic, diastolic dysfunction, left ventricular hypertrophy and consequently clinical heart failure (Tan et al., 2020). Our results showed that oxymatrine treatment significantly decreases TG, TC and LDL and increased HDL level in a dose dependent manner in diabetic animals. Documented report showed that decreased the endogenous production and absorption of triglyceride and cholesterol, and increase uptake in peripheral tissue (Calkin & Tontonoz, 2012), which might be the possible mechanism behind the anti-hyperlipidemic action of oxymatrine. Furthermore, oxymatrine high dose increased the expression of SIRT-1 which is associated with increased expression of AMPK leads to regulate the expression of PGC-1 $\alpha$  which is a master regulator of lipids in body (Cantó & Auwerx, 2009).

Interestingly, the current findings showed that oxymatrine has a protective effect in cardiomyopathy in the diabetic animals. Studies have reported that, in the starting stage of DCM diastolic dysfunction is occur which is followed by systolic dysfunction in the later stages (Schannwell, Schneppenheim, Perings, Plehn, & Strauer, 2002). Corroborated with the previous reports our findings showed that administration of STZ-nicotinamide increased the diastolic blood pressure followed by systolic blood pressure which indicate the systolic and diastolic dysfunction of the diabetic heart. However, results indicated that treatment with oxymatrine improved the systolic and diastolic blood pressure in a dose dependent manner.

Plethora of studies indicated that pathophysiology of DCM highlights that, alteration in the HW/BW ratio may occur due to the progressive loss of cardiac connective tissue in the damaged myocardium and HW/BW convolution layers are commonly used to calculate the hypertrophic index(Shiojima et al., 2005). Meanwhile, in our concurrent investigation, we observed a substantial increase in the HW/BW ratio in diabetic animals, which is similar to the previous study. While, treatment with oxymatrine as well as pioglitazone showed similar effect, indicating that our treatment regimen it has cardiac connective tissue and protective properties.

Beside this, STZ-nicotinamide induced diabetic rats showed impaired haemodynamic parameters, such as lower left ventricular maximum and minimum developed pressure which is evidence of contractile abnormalities, as well as diastolic dysfunction, as evidenced by raised LVEDP. We found that, treatment with pioglitazone, and oxymatrine treatment significantly decrease the LVEDP and increase the left ventricular maximum and minimum developed pressure, though among the different treatments oxymatrine high dose effective than pioglitazone and oxymatrine intermediate dose respectively as presented in the Fig. 6. these results indicate the cardioprotective action of the oxymatrine in the diabetic animals.

Several investigations indicated that in diabetic cardiomyopathy, the activity of LDH and CK-MB were increased due to myocardial dysfunction. Various reports showed that CKMB and LDH are known sensitive markers of damaged cardiomyocytes and there is a positive correlation found between damaged cardiomyocytes and myofibrillar breakdown (Aydin, Ugur, Aydin, Sahin, & Yardim, 2019). Similarly, in our study we found the increased serum LDH and CK-MB in STZ-nicotinamide induced diabetic rats. In contrast, treatment with oxymatrine significantly decreased the cardiac biomarker enzymes dose dependently in the serum of diabetic animals.

Cascade of alteration showed the increased ROS generation and oxidative damage are linked to the coexistence of hyperglycemia and hyperlipidemia(Lastra, Manrique, & Hayden, 2006). Concurrent research showed that pathogenesis and progression of DCM are linked to oxidative stress caused by hyperglycemia/hyperlipidemia(Liu, Wang, & Cai, 2014).Increased in ROS production leads to diminished the antioxidant capacity that accompanied with oxidative stress in diabetic heart(Rolo & Palmeira, 2006). Moreover, previous studies indicate that increased production of ROS is linked with the activation of pro-inflammatory cytokines and apoptotic pathway in cardiomyocytes (Wu et al., 2018). Consistent with preceding study, diabetic rat heart showed an rise in MDA and lower GSH and antioxidant enzyme activity

(Sabahi, Khoshnoud, Hosseini, Khoshraftar, & Rashedinia, 2021). Lipid peroxidation can weaken the phospholipid bilayer and render membrane-bound receptors and enzymes inactive, increasing cellular permeability and hastening cell death. Consequently, cellular redox balance provides a successful method to reduce oxidative stress in a number of disorders. In our investigation, diabetic rats exhibit higher level of lipid-peroxidation with lower levels of GSH and Catalase. We found that treatment with pioglitazone, oxymatrine significantly decreased the lipid-peroxidation marker level and increase the GSH and CAT levels. Moreover, nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor, activation of which leads increase the anti-oxidant defence mechanism, mitochondrial biogenesis and metabolic regulation (Vomhof-Dekrey & Picklo, 2012). Study of Jin et al., (2021) indicate that oxymatrine increase the expression of Nrf2 in pyroptosis (Jin et al., 2021). Similarly, our investigation showed that the expression of Nrf2 is downregulated in the diabetic rat's heart, similarly treatment with pioglitazone, oxymatrine intermediate and high dose significantly upregulates the expression of Nrf2, our findings indicate that oxymatrine high dose significantly upregulate the Nrf2 level than other treatments. From the above evidences we can confirmed that the anti-oxidant activity of oxymatrine due to upregulation of Nrf2 in cardiomyocytes that leads to decrease the ROS formation, metabolic dysregulation and mitochondrial dysfunction.

Numerous research have demonstrated a significant relationship between apoptosis, oxidative stress and inflammation in relation to diabetes.. Moreover, increased in ROS production and hyperlipidemia are reported to activate the inflammatory cytokines in diabetic heart (Oguntibeju, 2019). As a result, methods to lower the production of pro-inflammatory cytokines may directly benefit cardioprotection in diabetes. In our present study, we observed that diabetic animals showed increased level of TNF- $\alpha$  and IL-1 $\beta$ , on the other hand treatment with pioglitazone, oxymatrine reduced the level of TNF- $\alpha$  and IL-1 $\beta$  in a dose dependent manner. Furthermore, we observed that oxymatrine at high dose can markedly attenuates the inflammatory cytokines production than other treatments.

However, oxymatrine treatment modulates the pro-apoptotic expression in the DCM. Various reports showed that several mechanisms are involved in triggering apoptosis in the diabetic heart (RR). Briefly, dyslipidemia, hyperglycemia excessive ROS generation, inflammation and mitochondrial dysfunction are involved in pathophysiology of diabetic heart (Volpe, Villar-Delfino, Dos Anjos, & Nogueira-Machado, 2018). Herein, diabetic rats exhibit increased level of caspase3 which promotes apoptotic pathway by activation of caspase-3-activated DNase (CAD) which results in fragmentation of DNA (Gao et al., 2015). Findings of our study showed that oxymatrine treatment significantly decreased the caspase3 activity in a dose dependent manner.

SIRT1 is a nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase that removes acetyl groups from proteins. SIRT1 modulate different proteins related to hyperglycemia and produce anti-oxidant, anti-inflammatory and anti-apoptotic effect (Karbasforooshan & Karimi, 2017). Moreover, it has been reported that, activation of SIRT1 leads to increase the expression of AMPK and PGC-1 $\alpha$  which is a master regulator of lipid metabolism, oxidative stress, inflammation, apoptosis and gluconeogenesis (Zhang, Wang, Yang, Yang, & Ma, 2021). In the present study, there was significant downregulation of SIRT1

activity observed in the heart of DCM rats. While, treatment with oxymatrine significantly improved SIRT1 expression. Interestingly, our results showed that oxymatrine treatment at the high dose showed more profound effect in DCM animals.

Histopathological studies of the cardiac tissue of hyperglycemic rats revealed that diabetes is the key cause of abnormal structural changes such as myocardial degeneration, interstitial fibrosis, immune infiltration and necrosis (Al-Rasheed et al., 2017). These abnormalities are responsible for elevated oxidative stress, inflammation, deposition of collagen and apoptosis. One of the mechanisms of myocardial fibrosis is the activation of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) which further follows the smad pathway and leads to deposition of collagen in the cardiomyocytes and results in left ventricular hypertrophy (Yue, Meng, Pu, & Zhang, 2017). In our investigation, STZ- nicotinamide induced diabetic rats showed a remarkable increase in TGF- $\beta$ 1 expression, which was clearly observed in the histopathological study with increased myocardial fibrosis, immune infiltration and necrosis with disrupted cardiac architecture as shown in Figs. 13 and 14. Results from the various treatment groups indicated that pioglitazone, oxymatrine significantly improved the myocardial fibrosis, immune infiltration and restored the normal morphology of the cardiomyocytes with normal cellular nuclear shape, cytoplasm and cell boarder. However, all these protective effects are more remarkable in oxymatrine high dose group than other treatment group.

## Conclusion

The current investigation collectively provides the experimental proofs that oxymatrine high dose ameliorates against DCM in STZ-nicotinamide induced diabetic rats with a fundamental molecular mechanism involved in suppressing oxidative stress, inflammation, metabolic dysregulation, haemodynamic parameters, apoptosis and fibrosis through subsequently increase the expression of SIRT1/Nrf2 and decrease the expression of TGF- $\beta$ . Our study demonstrates that oxymatrine at the dose of (150 mg/kg, i.p) have the same potential to produce anti-diabetic effect as the standard pioglitazone but it showed efficacy in cardio-protection. Thus, in-order to treat or prevent DCM, oxymatrine has therapeutic promise. Our research, however, is restricted to the preclinical stage, and additional research is required to establish the safety limits and more thorough mechanism underlying the therapeutic potential of oxymatrine.

## Declarations

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### Ethical Approval

Animals: All the experimental animal protocols used in this research work were performed accordance with the regulations specified by the Institutional Animal Ethics Committee (IAEC), ISF college of pharmacy, Moga, Punjab. The experimental protocol were strictly followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India (Reg no.816/PO/ReBiBt/S/04/CPCSEA).

### Competing interests

The authors declare no competing interests.

### Authors' contributions

AS; developed the concept and methodology and supervised this research. SS and BJD conducted the experiments, and wrote the initial draft of the manuscript. SM and GG; carried out statistical analyses of the data

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Not applicable

### Availability of data and materials

The data will be made available upon reasonable request.

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## Tables

**Table.1 Effect of OMT on systolic and diastolic blood pressure**

Groups	Systolic blood pressure(mm of Hg)	Diastolic blood pressure(mm of Hg)
NORMAL	125.7±2.231	85.17±2.151
DIABETIC	164.8±3.198*	145.8±2.262*
D+PIO	131.2±1.579 <sup>#</sup>	98.83±5.522 <sup>#</sup>
D+OMT1	155.2±5.121 <sup>\$</sup>	134±4.235 <sup>\$</sup>
D+OMT2	145.5±2.646 <sup>#\$@</sup>	115.8±3.060 <sup>#\$@</sup>
D+OMT3	133.7±1.745 <sup>#\$@%</sup>	98±1.932 <sup>#\$@%</sup>

Values are expressed as means ± SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and <sup>#</sup> $P < 0.05$ , when compared to the diabetic group, <sup>\$</sup> $P < 0.05$ , when compared to the D+PIO group, <sup>@</sup> $P < 0.05$  when compared to the D+OMT1 group, <sup>%</sup> $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+PIO, diabetic group treated with pioglitazone (10 mg/kg, *p.o*); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, *i.p.*); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, *i.p.*); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, *i.p.*).

**Table 2: Effect of OMT on histopathological changes**

Histopathological findings	NORMAL	DIABETIC	D+PIO	D+OMT 1	D+OMT 2	D+OMT 3
Disrupted morphology with abnormal nuclei, cytoplasm and cell boarder	0	###	#	###	##	0
Inflammatory cells infiltration	0	###	#	###	##	0
Necrosis	0	##	0	##	#	0

The routine H&E stained section changes in rat myocardium were scored and graded into four grades, i.e., absent (0), mild changes (#), moderate changes (##), severe changes (###)

## Figures

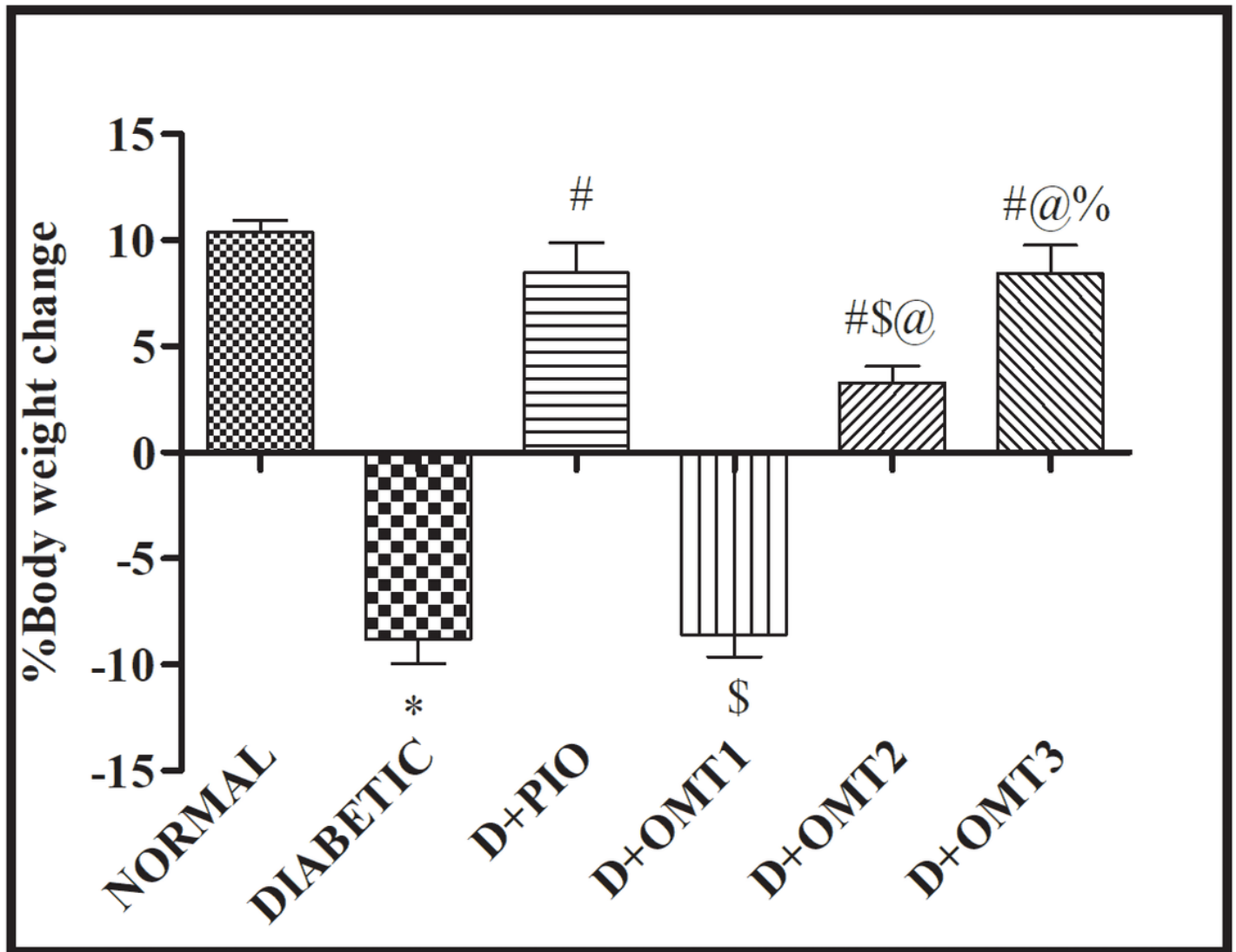


Figure 1

### Effect of OMT on % body weight change

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1, % $P < 0.05$  when compared to D+OMT2 (One-way ANOVA followed by Newman-Keuls test). D+PIO, diabetic group treated with pioglitazone (10 mg/kg, *p.o*); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, *i.p.*); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, *i.p.*); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, *i.p.*).

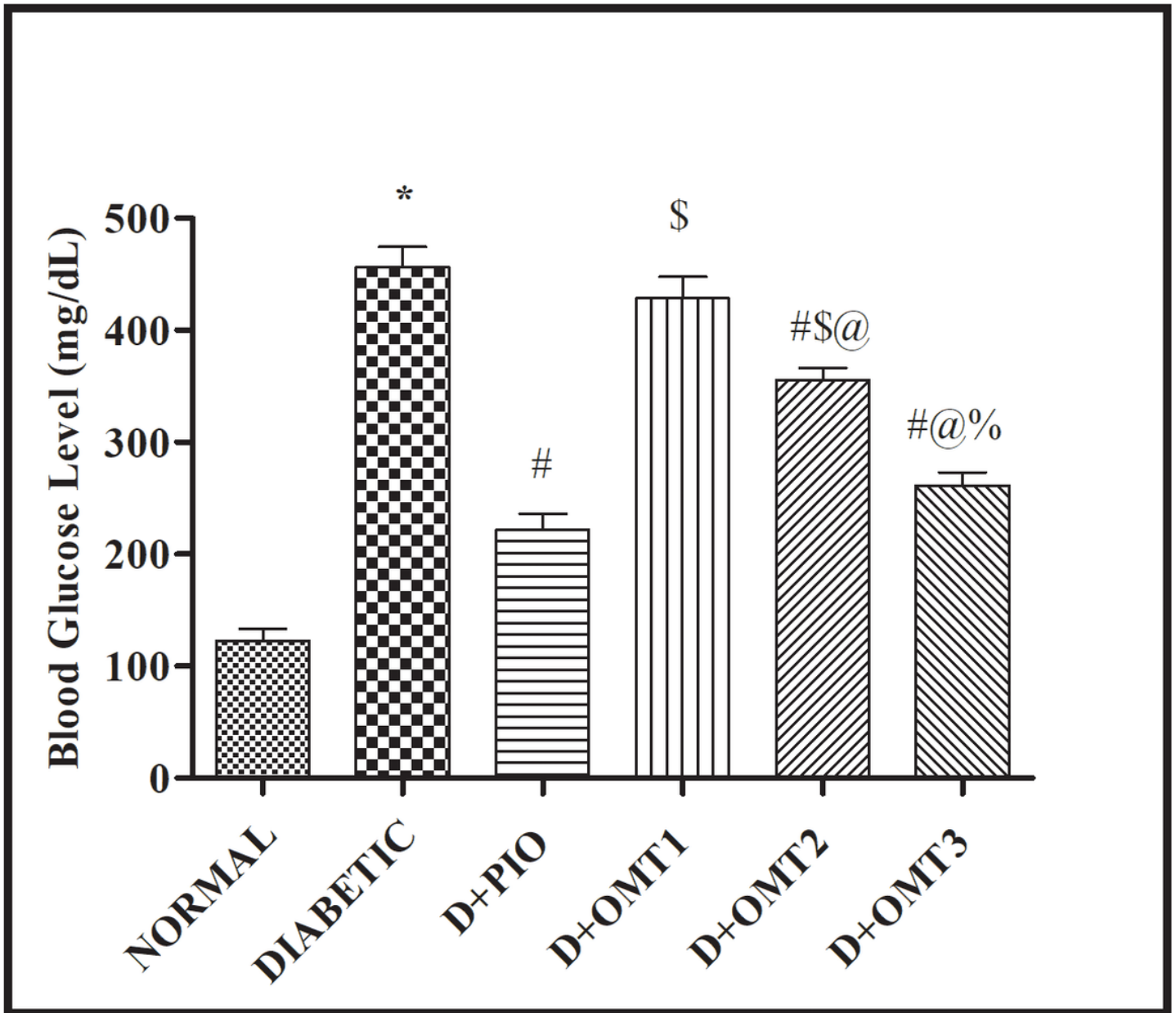


Figure 2

**Effect of OMT on blood glucose level**

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1, % $P < 0.05$  when compared to D+OMT2 (One-way ANOVA followed by Newman-Keuls test). D+PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).

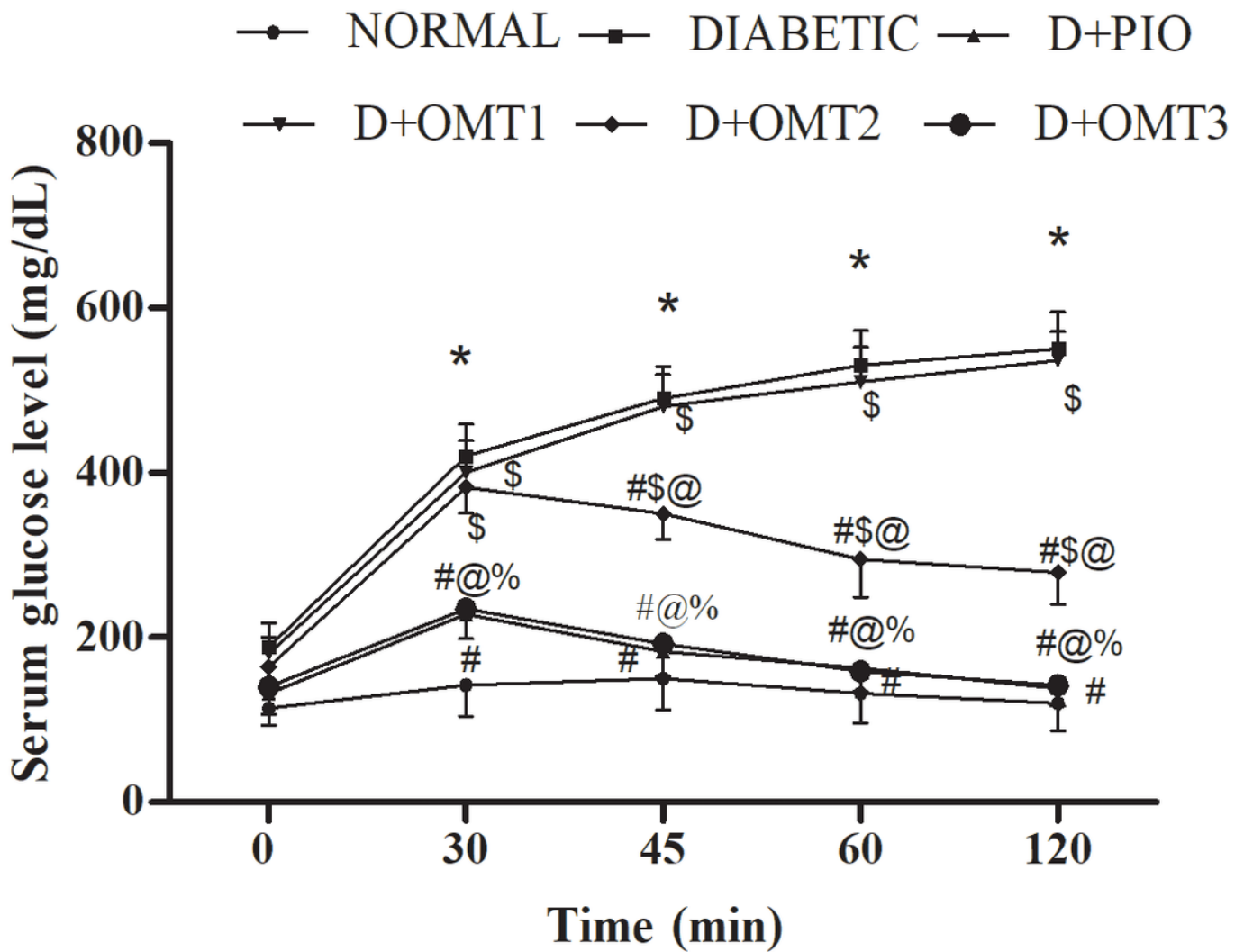
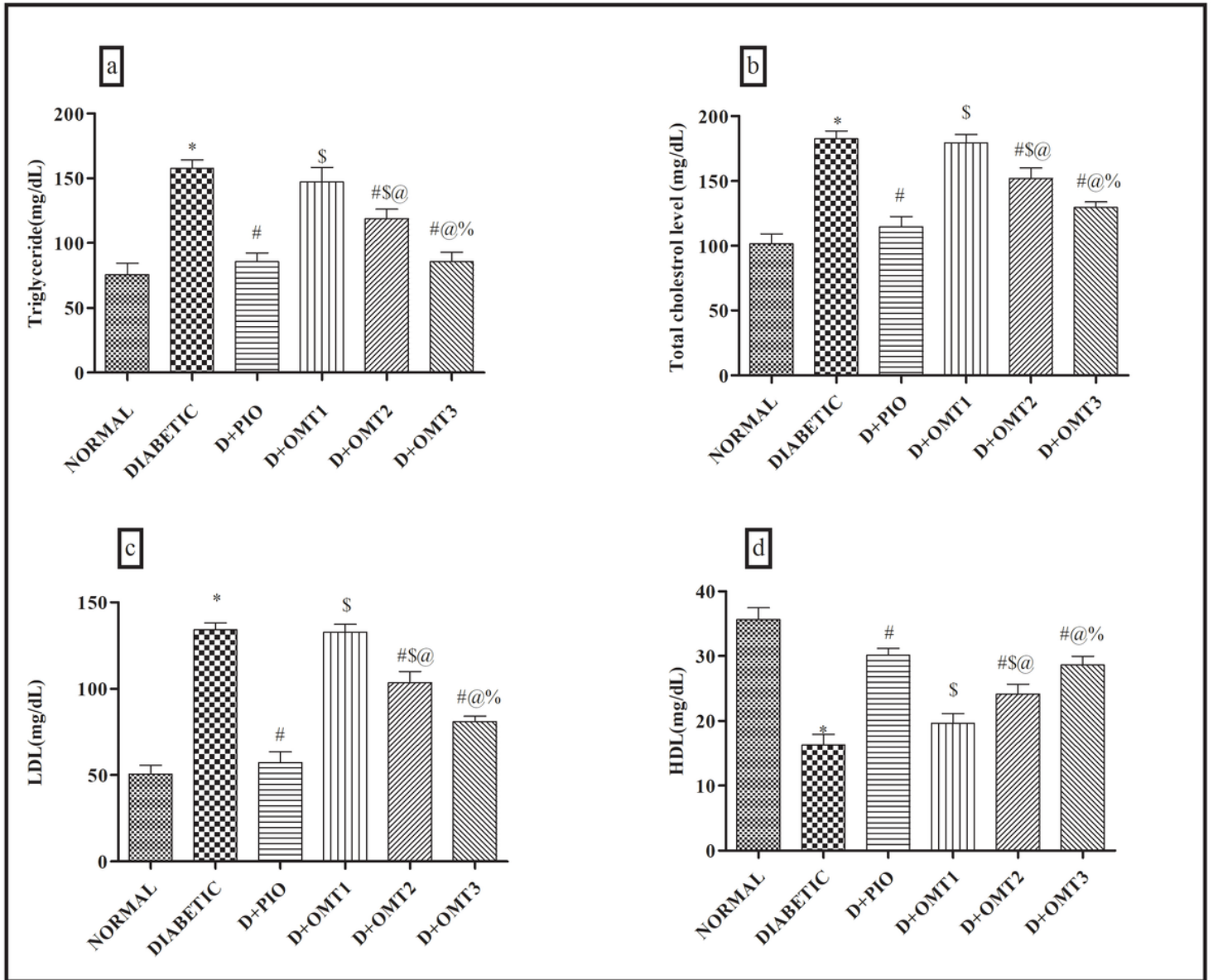


Figure 3

Effect of OMT on OGTT

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1, % $P < 0.05$  when compared to D+OMT2 (Two-way ANOVA followed by Bonferroni post hoc test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).



**Figure 4**

**Effect of OMT on serum lipid profile**

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1, % $P < 0.05$  when compared to D+OMT2 (One-way ANOVA followed by Newman-Keuls test). D+PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).



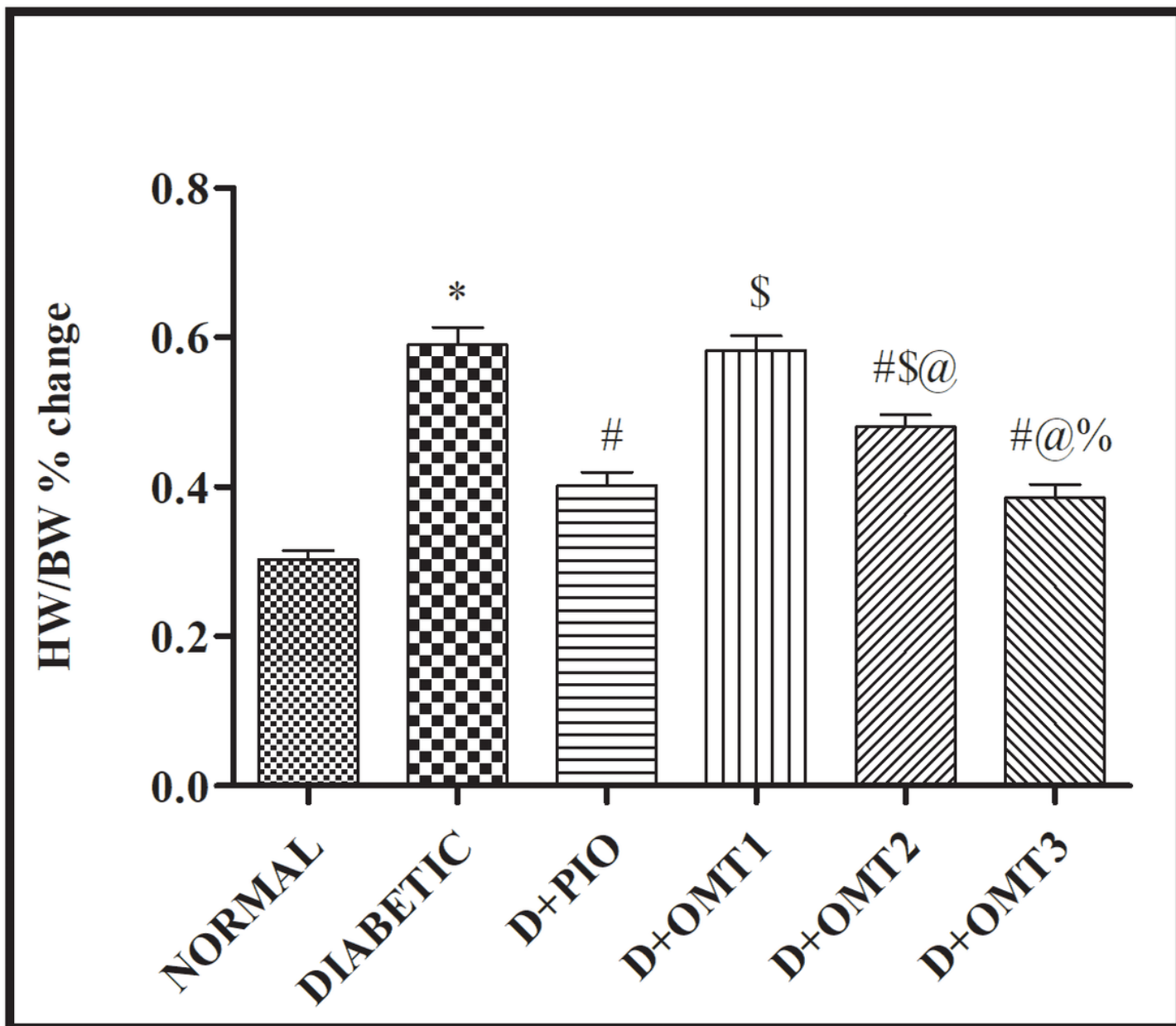


Figure 5

**Effect of OMT on heart weight/body weight ratio (HW/BW)**

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, *p.o*); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, *i.p.*); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, *i.p.*); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, *i.p.*).

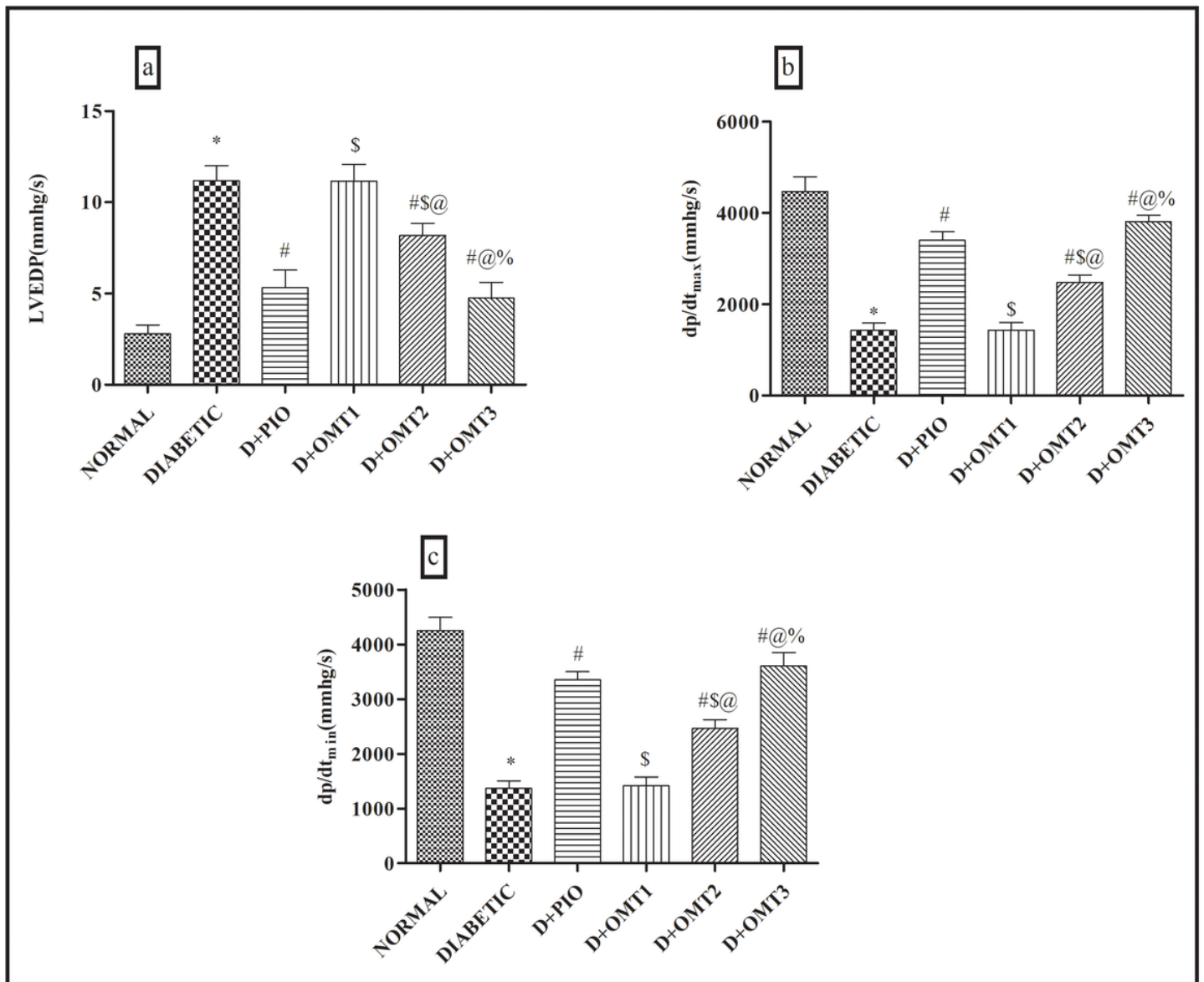


Figure 6

### Effect of OMT on hemodynamic parameters of heart

Values are expressed as means  $\pm$  SEMs ( $n = 8$ ); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+PIO, diabetic group treated with pioglitazone (10 mg/kg, *p.o.*); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, *i.p.*); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, *i.p.*); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, *i.p.*).

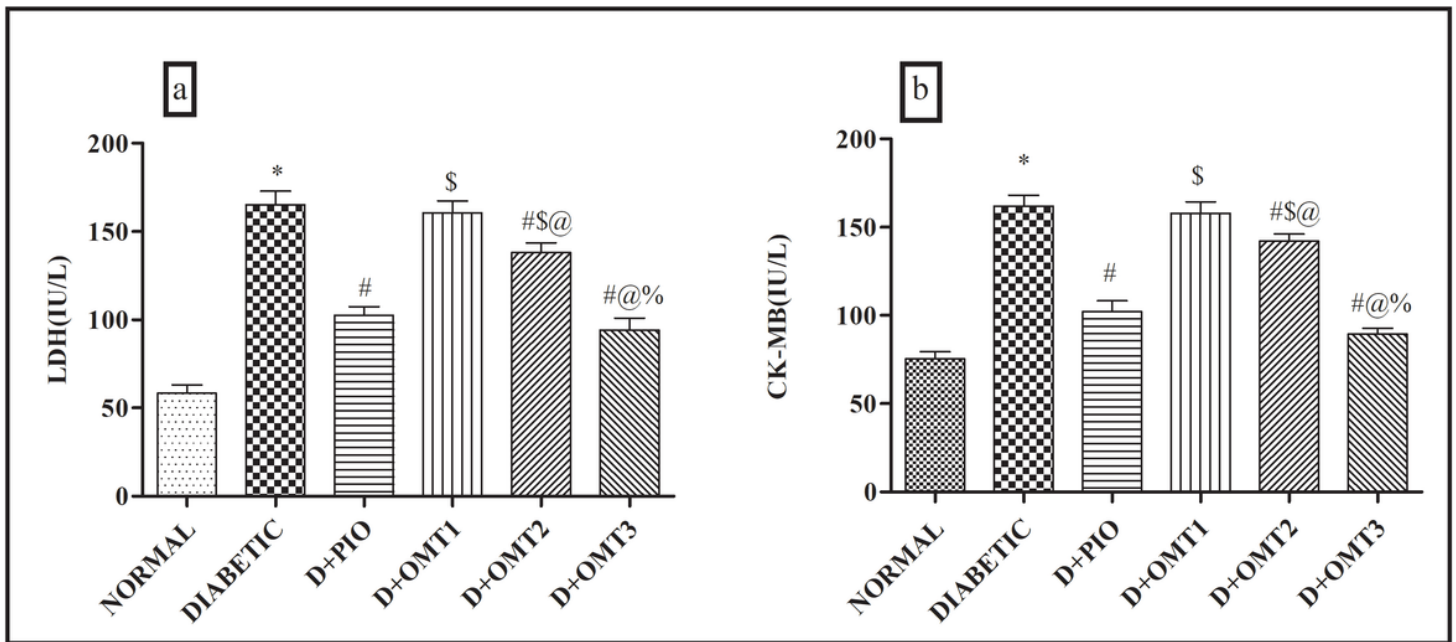
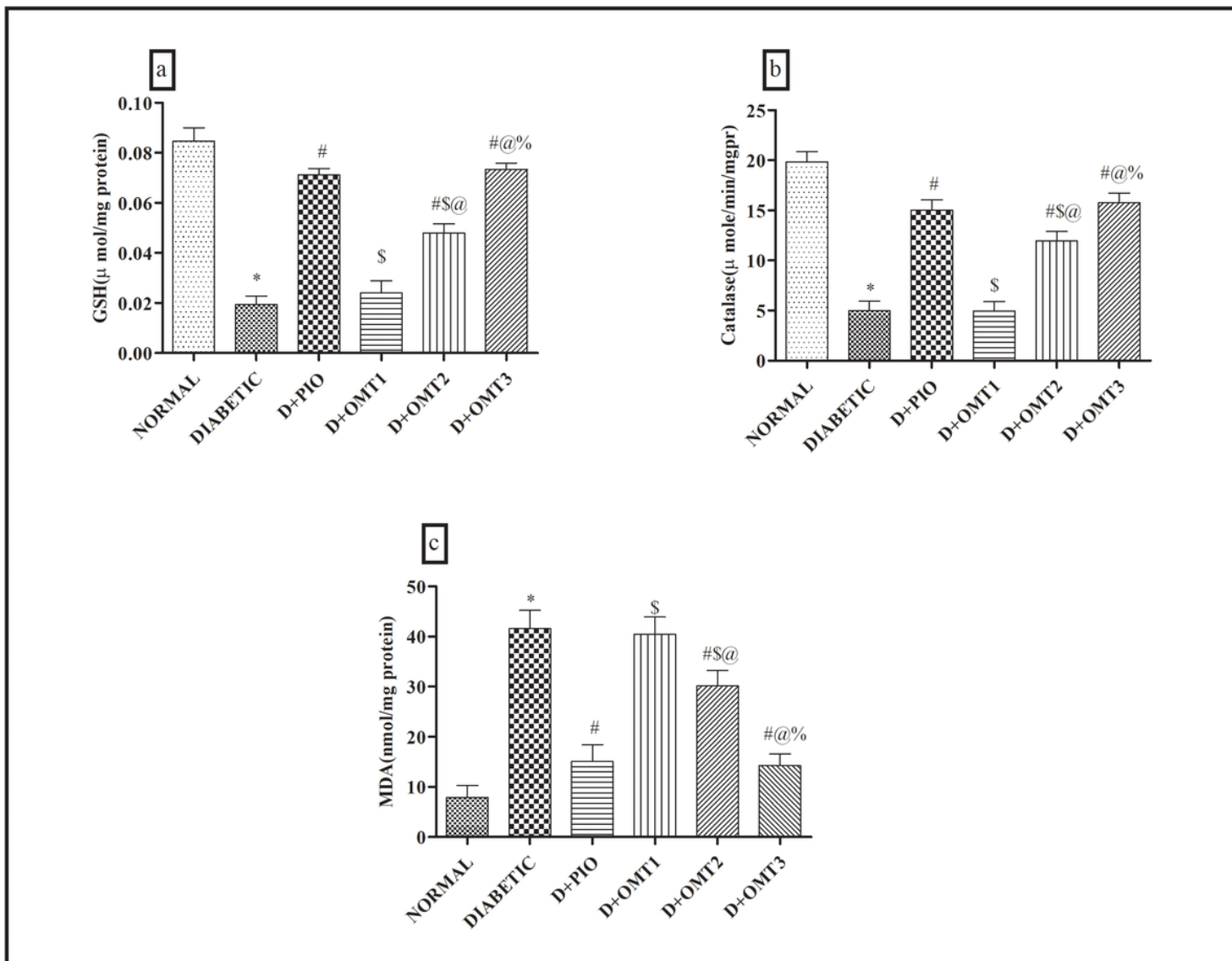


Figure 7

### Effect of OMT on serum cardiac marker enzymes

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, *p.o*); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, *i.p.*); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, *i.p.*); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, *i.p.*).



**Figure 8**

**Effect of OMT on oxidative stress biomarkers**

Values are expressed as means ± SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).

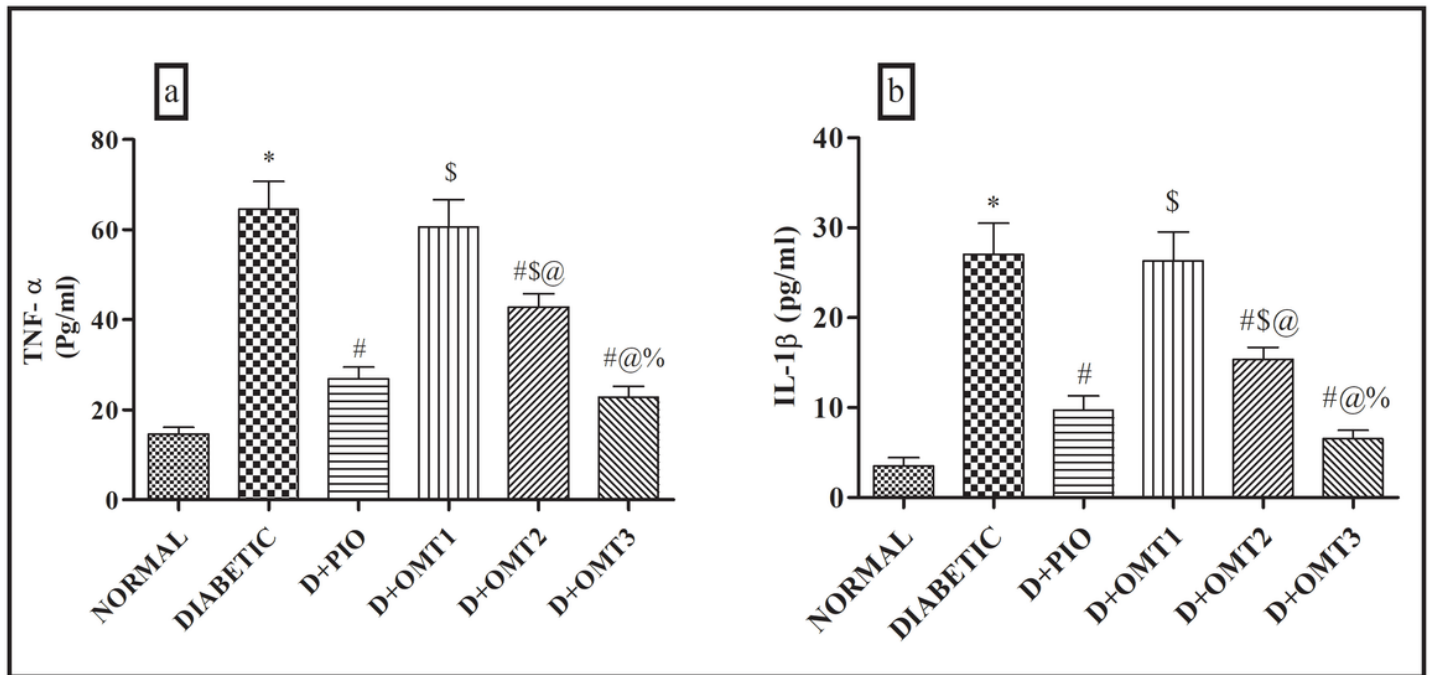


Figure 9

### Effect of OMT on inflammatory cytokine

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).

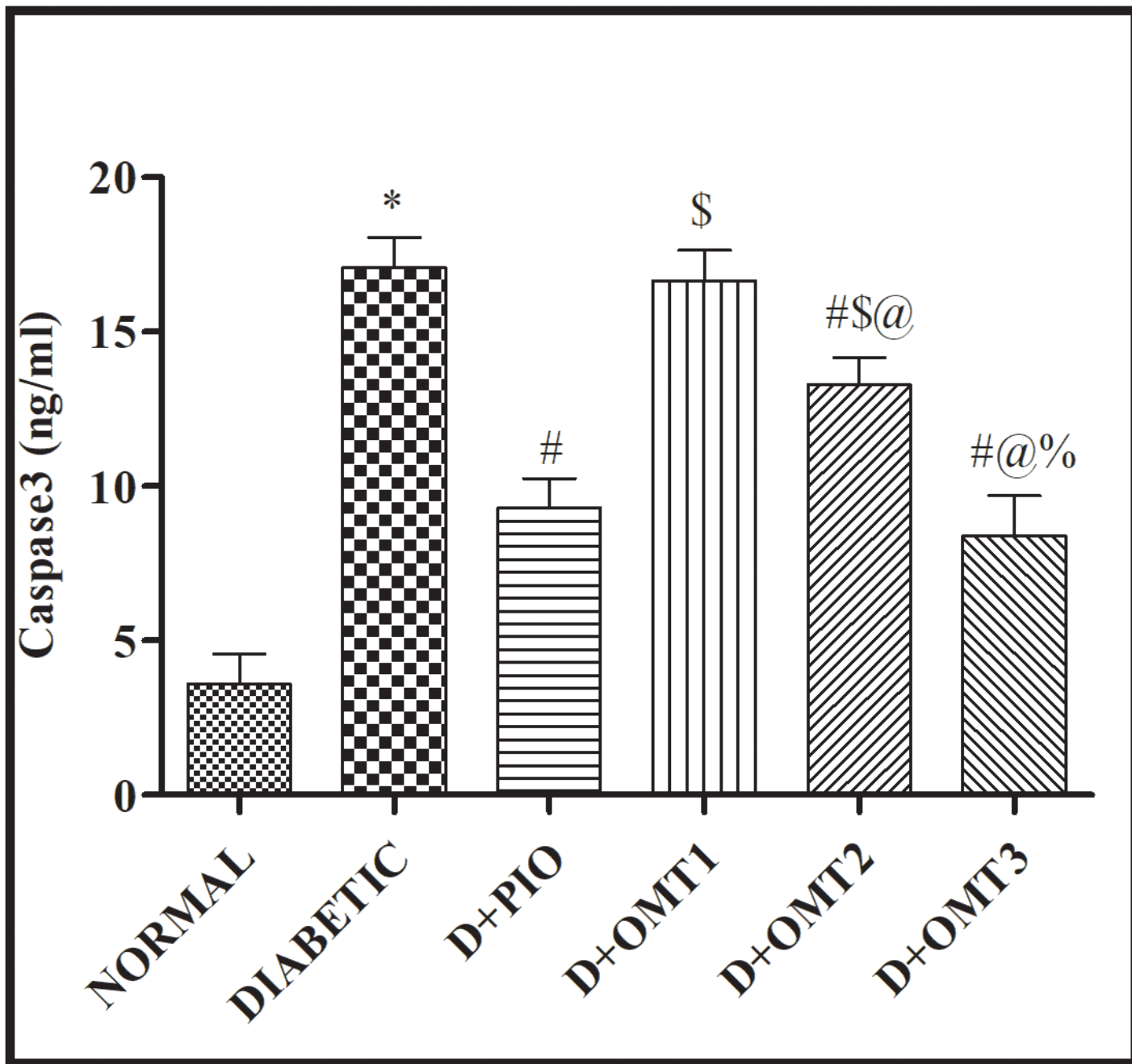


Figure 10

### Effect of OMT on caspase3 activity

Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).

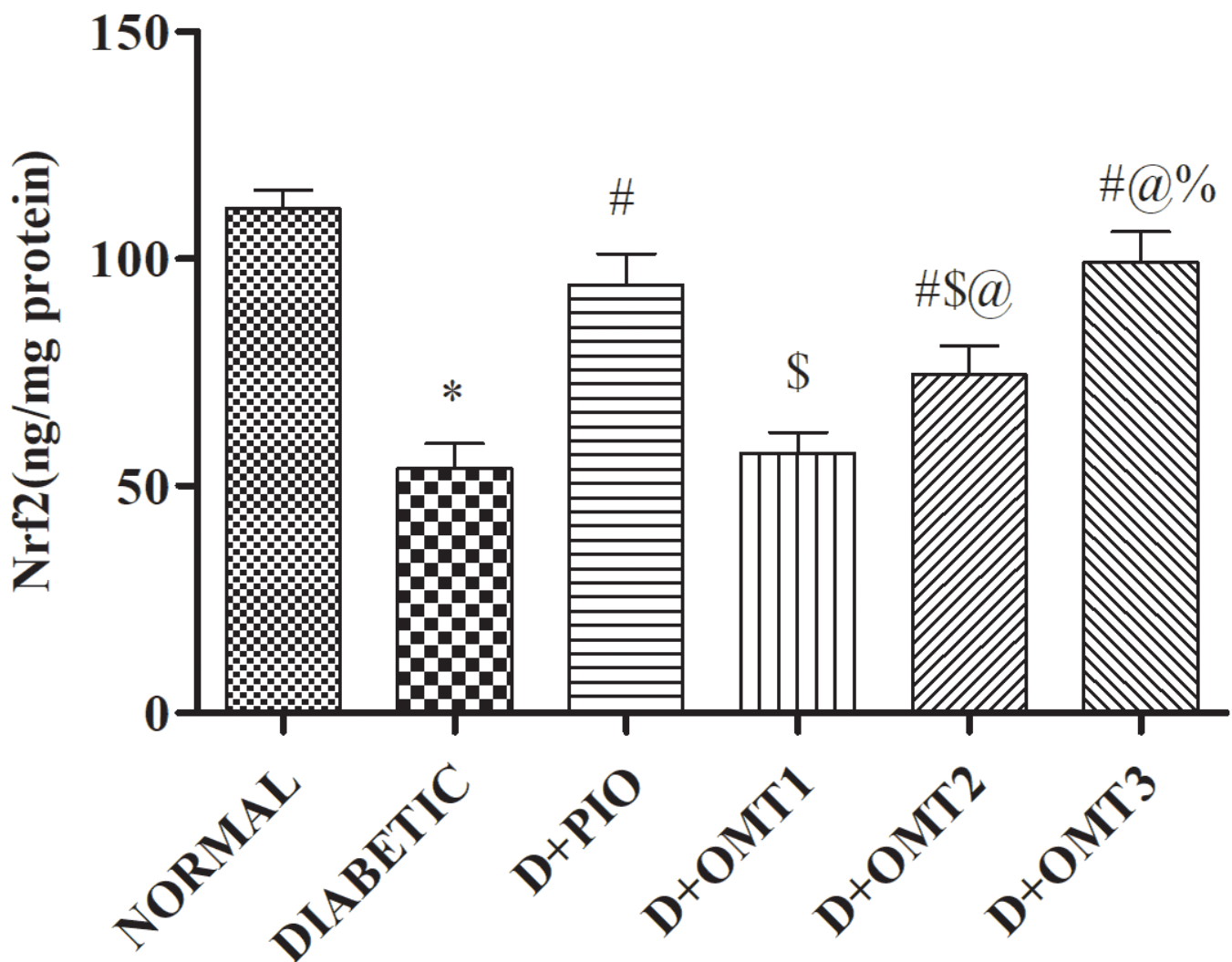
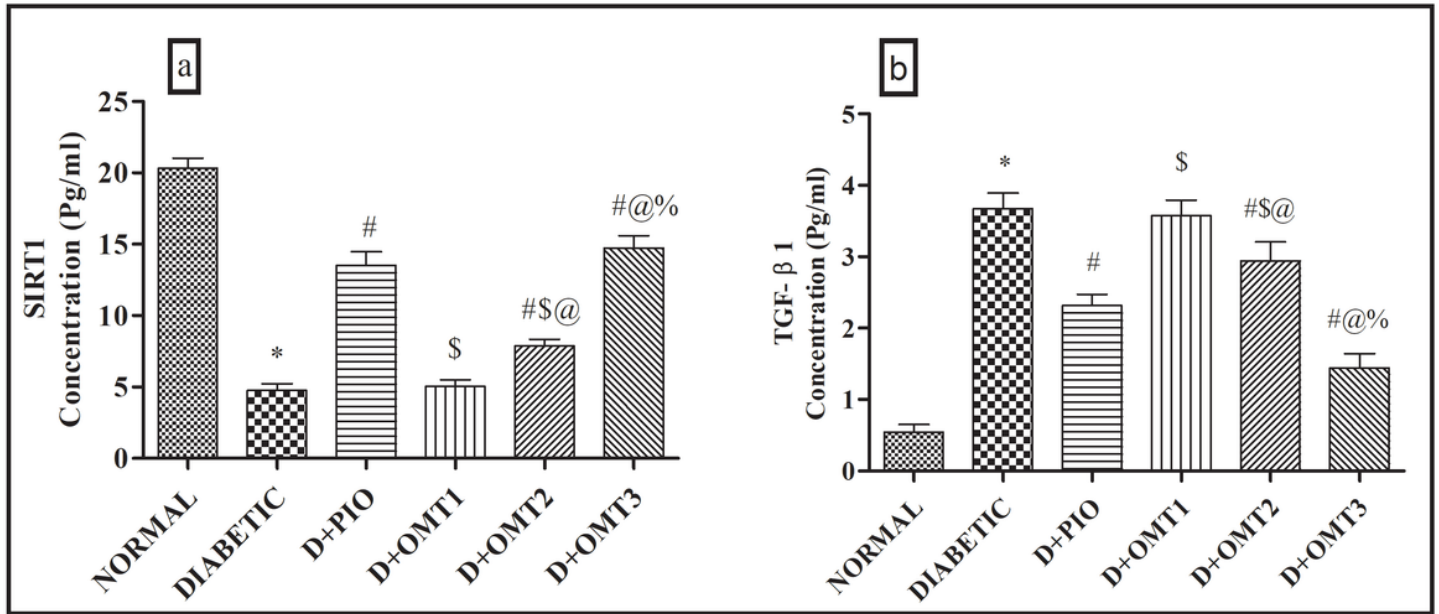


Figure 11

### Effect of OMT on Nrf2 expression

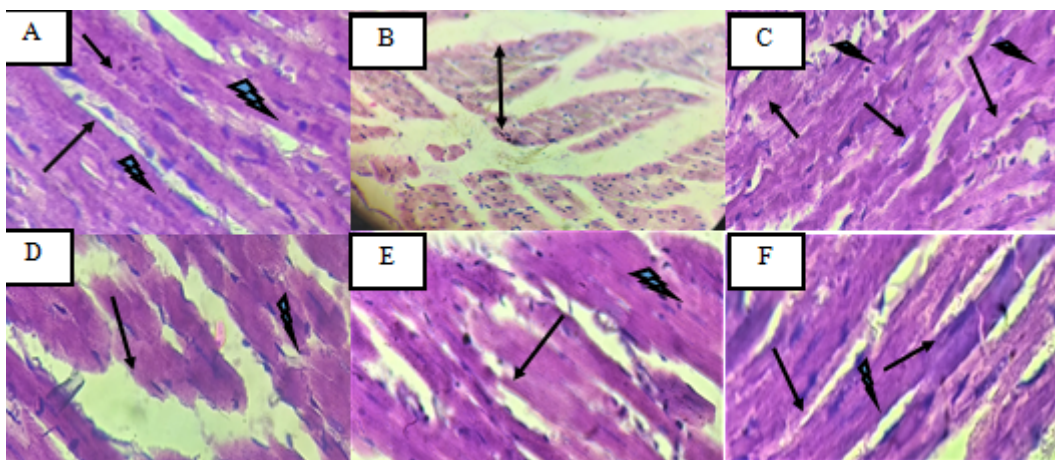
Values are expressed as means  $\pm$  SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).



**Figure 12**

**Effect of OMT on SIRT1 and TGF-β1 expression**

Values are expressed as means ± SEMs (n = 8); \* $P < 0.05$ , when compared to the normal and # $P < 0.05$ , when compared to the diabetic group, \$ $P < 0.05$ , when compared to the D+PIO group, @ $P < 0.05$  when compared to the D+OMT1 group, % $P < 0.05$  when compared to D+OMT2 group (One-way ANOVA followed by Newman-Keuls test). D+ PIO, diabetic group treated with pioglitazone (10 mg/kg, p.o); D+OMT1, diabetic group treated with oxymatrine (25 mg/kg, i.p.); D+OMT2, diabetic group treated with oxymatrine (100 mg/kg, i.p.); D+OMT3, diabetic group treated with oxymatrine (150 mg/kg, i.p).

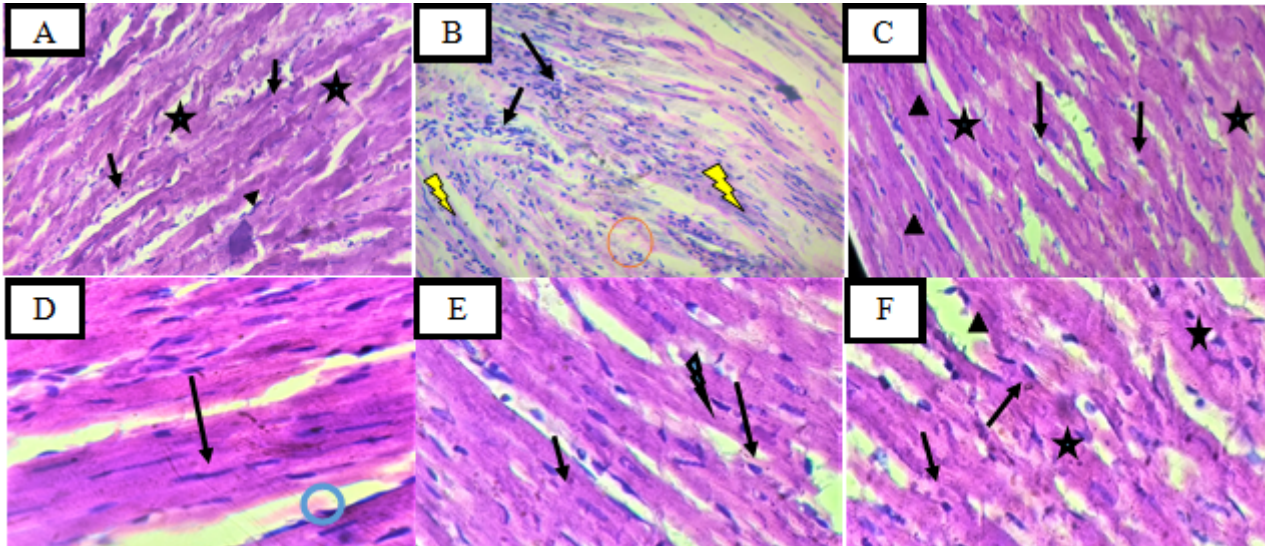


**Figure 13**

**Effect of OMT on the cardiac fibrosis (haematoxylin and eosin staining of heart sections in various group) (H&E ×40X)**



Section of the cardiac tissue from (A) the normal group showing the cardiac muscle fibers without any collagen deposition(zigzag) and interstitial fibrosis(arrow) (H&E stain×400). (B) diabetic group showing collagen deposition(triangle) and myocardial fibrosis (bi-directional arrow).(C) Diabetic group treated with pioglitazone (10 mg/kg, p.o)showing preserved architecture of myocardium with reduced myocardial fibrosis(arrow) and collagen deposition(zigzag). (D) Diabetic group treated with oxymatrine (25 mg/kg, i.p) showing no effect in collagen deposition(zigzag) and myocardial fibrosis(arrow). (E) diabetic group treated with oxymatrine (100mg/ kg, i.p) showing mild effect in reduction of myocardial fibrosis(arrow) and collagen deposition. (F) Diabetic group treated with oxymatrine (150 mg/kg, i.p) showing restoration of architecture of myocardium with reduced myocardial fibrosis(arrow) and collagen deposition(zigzag).



**Figure 14**

**Effect of OMT on cardiac morphological damage (haematoxylin and eosin staining of heart sections in various group (H&E ×40X):**

(A) Representative photomicrograph of the heart of the normal group showing normal cardiomyocytes which appeared as striated muscle fibers(star) with normal nuclei (arrows), normal cytoplasm with distinct cell borders (triangle). (B) Representative photomicrograph of the heart of the diabetic group showing inflammatory cells infiltration(arrow), pyknotic cells with disrupted cytoplasm(zigzag) and necrosis (circle). (C) Diabetic group treated with pioglitazone (10 mg/kg, p.o) showing reduced inflammatory cell infiltration(star) and restoration of normal appearance of myocardium with normal nuclei (arrow), cytoplasm(triangle). (D) Diabetic group treated with oxymatrine (25 mg/kg, i.p) showing no effect in inflammatory cell infiltration (zigzag), necrosis(circle) and morphology (arrow) of cardiac cells. (E) Diabetic group treated with oxymatrine (100mg/ kg, i.p) showing mild effect in reduction of inflammatory cells infiltration (zigzag) and recover of nuclei and cytoplasm (arrow). (F) Diabetic group treated with oxymatrine (150 mg/kg, i.p) showing ameliorative effect in inflammatory cells infiltration (star), normal cytoplasm(triangle) and normal nuclei(arrow).