International Standard Serial Number: 0976-139X

Deccan J. of Pharmacology 1(2): April-June 2010

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

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PIOGLITAZONE REDUCES ON EXPERIMENTALY INDUCED MYOCARDIAL INFARCTION IN DIABETIC RATS

Jagdish Kakadiya*, Divyang Patel, N. J. Shah

Pharmacology Department, Dharmaj Degree Pharmacy College, Petlad-Khambhat Road, Dharmaj, Anand-388430, Gujarat, INDIA.

ABSTRACT

Present study was designed to evaluate Pioglitazone on cardiac complication in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Pioglitazone (10 mg/kg, p.o, PIO) was administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and nicotinamide (110 mg/kg, i.p, NIC) and after isoproterenol (200mg/kg, s.c., ISO) induced myocardial infarction in rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and glycogen and nitrite carried out for further estimations. Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c), creatine kinase (CK), Glutamate oxaloacetate transferase (GOT), glycogen and nitrite whereas the levels of myocardial infarct size was found low to be significant ($p<0.05$).). The myocardial infarction in diabetic rats also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats. The PIO treated diabetic rats exhibited reduction in necrosis with less fragmentation of fibres as compared to diabetic control groups, which reflects the cardio

protective effect of PIO. This study concluded that PIO at 10 mg/kg may show reduce experimentally induced myocardial infarction in type 2 diabetic rats.

KEYWORDS: Pioglitazone, cardioprotective, isoproterenol, Type 2 diabetic, Histopathology

INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance [1, 2]. Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor [3], due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators [4]. Recently, a protective effect of pioglitazone against oxidative stress in liver and kidney of diabetic rabbits [5] has been reported.

PIO hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. PIO showed dose dependant beneficial effects in many of the pathological conditions including reduction in blood glucose, lowering blood pressure and restoring endothelial functions in animals [6]. Pioglitazone lowers blood pressure and restores blunted endothelium-dependent vasodilatation in fructose-fed rats [7], insulin-resistant rheus monkey [8], SHR [9] and sucrosefed SHR [10].

So far the effect of pioglitazone on experimentally induced myocardial infarction in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to instigate the effect of pioglitazone treatment on serum heart marker, heart tissue parameter and histopathological alteration in Isoproterenol Induced myocardial infarction in type 2 diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Pioglitazone hydrochloride was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (210 ± 15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitium.*

Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats or mice followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose [11]. Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study. Pioglitazone (10mg/kg, p.o) was administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and carried out for further estimations.

Experimental Protocol

Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (**ND-CON**)] and normal saline subcutaneously on 29th and 30th day.

Group 2: Non-diabetic control treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-PIO**)] and normal saline subcutaneously on 29th and 30th day.

Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (**D-CON**)] and received ISO (200mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 4: STZ-NIC diabetic rats treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-PIO**)] and received ISO (200mg/kg, s.c.) on 29th and 30th day in normal saline.

BIOCHEMICAL ESTIMATIONS

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of Serum Markers

On 4th week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose, HbA1c, CK and GOT were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). *In vitro* quantitative determination of the activity of myocardial glycogen and myocardial nitrite [12] levels.

Histological Examination

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 10% buffered formalin. Hearts which were stored in 10% formalin were embedded in paraffin, sections cut at 5 µm and were stained with haematoxyline and eosin. The sections of the heart were observed under microscope (Olympus BX10) for histological changes.

Infarct size measurement

The suture was tired again. The myocardial infarct size was measured by injecting evans blue solutions (2 % in PBS) retrograde through aorta to area at risk (AAR). The heart was frozen and about 6-8 thin sections were cut (approx 1-1.5 mm) from apex to base. The sections were placed in triphenyl tetrazolium chloride (TTC) solution (1% in PBS, pH 7.4) and kept at 37 ^{o}C for 20 min. The sections were fixed in 10 % formal saline overnight; both sides of slides of slices were scanned with scanner for measurement of AAR and infarct size (IS) by image J Software (1.30v). The area free from blue staining was area at risk. The portion stained red colour was salvaged myocardium and stained whitish portion was infarct size. Each area i.e. total, AAR, IS

of each image was measured five times and the measured five times and the mean of each area of every slide was calculated. This was done to ensure minimum error in measurement of each area. The complete area of all the sections in each slide was added to get total area. The zone free from blur stain of each section from the slide was added to get the AAR. It was calculated as percent of total area, while IS was calculated as percent of AAR.

Statistical Analysis

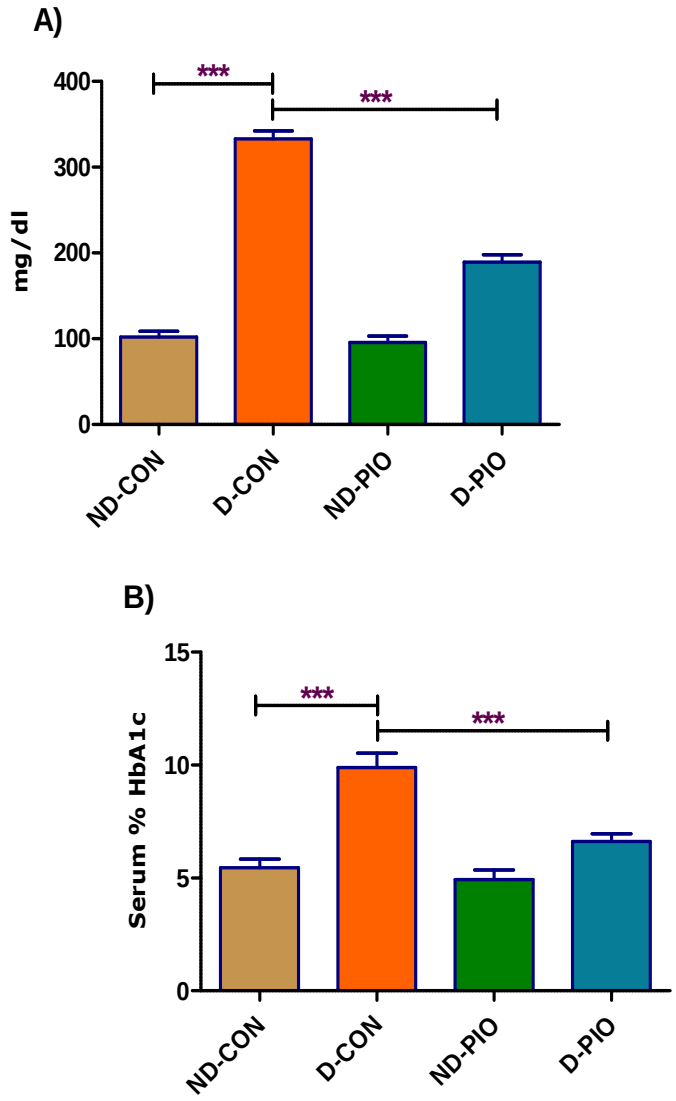
All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Characterization of Type 2 Diabetes

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Figure 1).

Figure 1. Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.001, ***P<0.001 considered statistically significant as compared to respective Control group.

Body Weight and Heart Weight

Final body Weight of control animals was no significant as compared to initial body weight. There was a significant reduction in final body weight as compared to initial body weight of D-CON diabetic group (Table 1). PIO treatment had no significant effect on the body weight of ND-CON Group but it prevents body weight loss in D-CON group animals. There was a significant ($p < 0.05$) increased in heart weight of diabetic rats (D-CON). PIO treatment could not prevent in heart weight in diabetic rats (Table 1).

Effect of PIO on serum enzymes

There was a significant ($p<0.05$) increase in CK and ($p<0.001$) increase in GOT level after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 1). Treatment of PIO in STZ-NIC diabetic rats (D-PIO) could not reduce elevated levels of serum CK and GOT (Figure 2).

Effect of PIO on myocardial tissue parameter

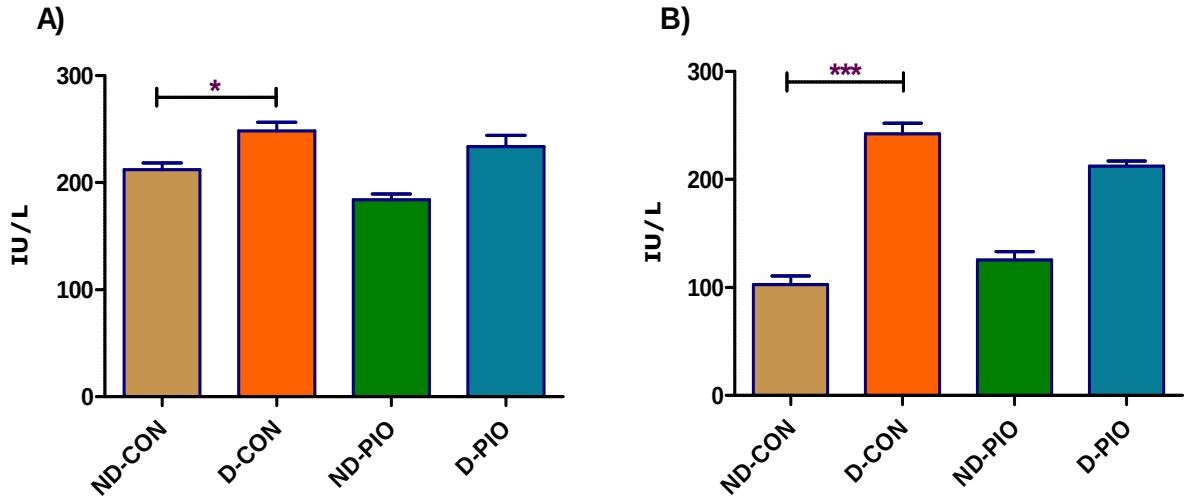
There was a significant ($P < 0.001$) increase in myocardial glycogen level in D-CON group as compared to ND-CON group after myocardial ischemia reperfusion injury. PIO treatment significantly ($P < 0.05$) reduced glycogen deposition in diabetic animal (D-PIO) as compared to diabetic control group (ND-PIO) (Figure 3A). There was a significant ($P < 0.01$) increase in myocardial nitrite level in D-CON group as compared to ND-CON group after myocardial infarction. PIO treatment significantly ($P < 0.05$) reduced nitrite level in heart in diabetic animal (D-PIO) as compared to diabetic control group (ND-PIO) (Figure 3B).

Table 1 Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in body weight, heart weight and heart to body weight ratio after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Groups	Body Weight						Heart Weight (gm)			Heart to Body Weight ratio		
	Initial			Final								
ND-CON	240.6	±	12.5	251.6	±	15.4	0.88	±	0.044	0.00349	±	0.00064
D-CON	249.2	±	17.4	224.4	±	16.1$^{\#}$	0.98	±	0.072*	0.00436	±	0.00027*
ND-PIO	237.5	±	18.4	245.6	±	12.4	0.86	±	0.047	0.00350	±	0.00029
D-PIO	239.1	±	16.8	250.2	±	16.4$^{\#}$	0.99	±	0.078*	0.00395	±	0.00046*

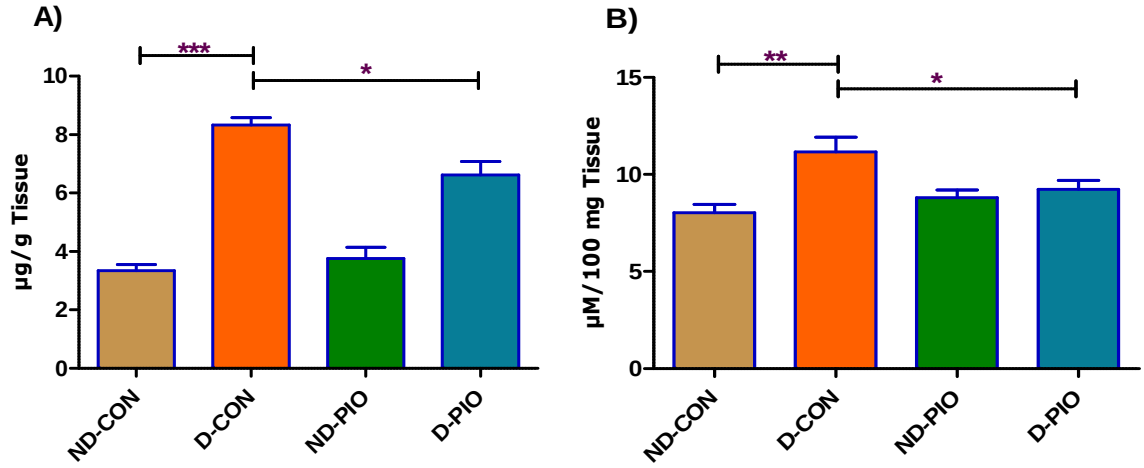
Values are expressed as mean ± SEM for six animals in the group. * $P<0.05$ compared to respective control group and # $P<0.05$ compared to initial weight.

Figure 2. Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in serum Creatine kinase (CK) and Glutamate oxalatoacetate transferase (GOT) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.001, ***P<0.001 considered statistically significant as compared to respective Control group.

Figure 3. Effect of Pioglitazone (10 mg/kg/day, p.o) on myocardial changes in Glycogen (A) and Nitrite (B) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.001, ***P<0.001 considered statistically significant as compared to respective Control group.

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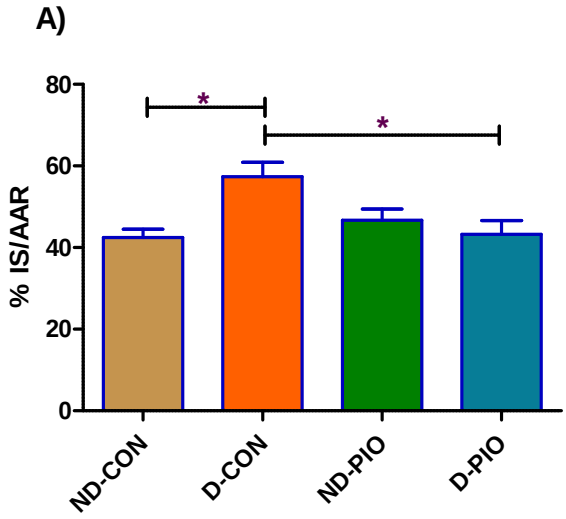
Figure 4. Effect of Pioglitazone (10 mg/kg/day, p.o) on myocardial infact size changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.001, ***P<0.001 considered statistically significant as compared to respective Control group.

Myocardial Infarct Size

There was a significant (P < 0.05) increase in infarct size after myocardial infarction in diabetic rats (D-CON) as compared to ND-CON. PIO treatment significantly (P < 0.05) reduced infarct size in D-PIO group as compared to D-CON group (Figure 4, 5). However, treatment with PIO could not reduce infarct size in non diabetic rats (ND-PIO) as compared to ND-CON group (Figure 4, 5).

Histopathology of Heart

The photomicrographs revealed that induction of myocardial infarction caused more necrotic damage along with focal loss and fragmentation of muscle fibres of myocardial in diabetic rats (D-CON) than non diabetic rats (ND-CON) (fig. 6). The myocardial infarction in diabetic rats (D-CON) also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats (ND-CON). The PIO treated diabetic rats (D-PIO) exhibited reduction in necrosis with less fragmentation of fibres as compared to D-CON groups, which reflects the cardio protective effect of PIO (Fig. 6). However, PIO treatment could not

protect myocardial infarction in non diabetic rats (ND-PIO) as it could not reduce neutrophil infiltration and cellular edema.

Figure 5. Effect of Pioglitazone (10 mg/kg/day, p.o) on TTC stained myocardial sections changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

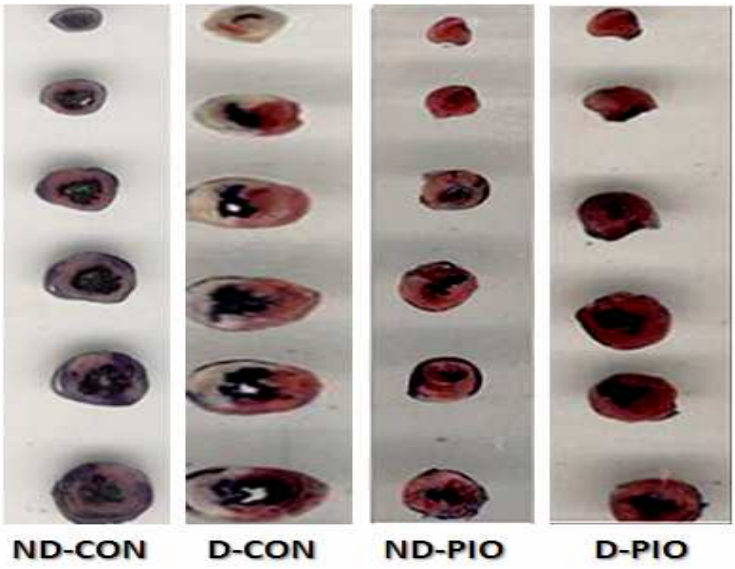
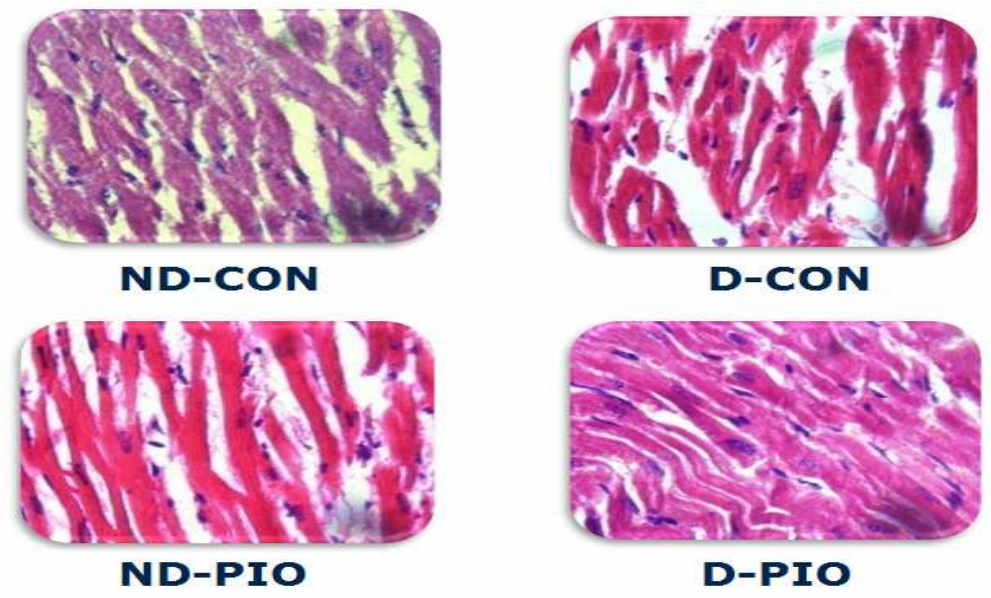


Figure 6. Effect of Pioglitazone (10 mg/kg/day, p.o) on light micrographs of histopathological section of heart changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.



DISCUSSION

The present study was under taken with the objective of exploring the Pioglitazone Reduces on experimently induced myocardial infarction in diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Peroxisome proliferator-activated receptors are nuclear transcription factors that play a role in insulin sensitivity [13].

In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. Significant decrease was observed in the glucose and HbA1c level in diabetic rats after treatment with PIO (10 mg/kg) when compared with D-CON rats at the end of experimental period. There was a significant increase in heart weight in STZ-NIC diabetic rats which may be due to cardiomyopathy associated with diabetes. It was reflected by increase in serum CK and GOT levels along with heart weight to body weight ratio. Pioglitazone could not protect the heart from cardiomyopathy associated with STZ-NIC diabetes. This may be the reason for elevated serum CK and GOT level in D-PIO group. However, Pioglitazone is reported for its detrimental [14] and protective [15] effects through its anti-inflammatory actions against heart failure but not yet in cardiomyopathy associated with STZ-NIC diabetes. The lack of α-tocopherol moiety which is responsible for the cardioprotective activity may be responsible for the failure of Pioglitazone to prevent cardiomyopathy in STZ diabetic rats [16].

Myocardial infarction causes further increase in oxidative stress and reduction in nitric oxide due to endothelial dysfunction. Pioglitazone reduced myocardial infarct size in STZ-NIC diabetic rats. The glycogen deposition in heart is increased in STZ-NIC diabetic rats which may be due to reduction in glucose utilization. PIO reduced cardiac glycogen content in STZ-NIC diabetic rats (D-PIO) by increasing glucose utilization after myocardial infarction. Therefore, another possibility for cardioprotection by PIO may be shifting of energy substrate metabolism from fatty acid to glucose.

There may be several mechanisms for cardioprotective by PIO against myocardial infarction. It may be due to improvement in NO availability, utilization of glucose in STZ-NIC diabetic rats.

Administration of STZ caused increase in serum CK, GOT and Pioglitazone (10 mg/kg, p.o) could reduce them. This study concluded that PIO at 10 mg/kg may show reduced on experimentally induced myocardial infarction in diabetic rats.

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