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In-vitro antioxidant activity in heart of pioglitazone on isoproterenol induced myocardial infarction in normal and Type-2 Diabetic rats

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ABSTRACT

Present study was designed to evaluate in vitro antioxidant activity in heart of Pioglitazone on isoproterenol induced myocardial infarction in normal and diabetic in rats. Pioglitazone (10mg/kg, p.o) 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 diabetic rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) heart tissue sample of each rat was collected and antioxidative parameter 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). At the end of experimental period the level of malondialdehyde formation/ lipid peroxidation (LPO) in liver tissue was significantly increased. Whereas, the activity of biomarkers of oxidative stress such as reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were found to be decreased significantly compared to control rats. Treatment with Pioglitazone significantly restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation in compared to diabetic control group. This study concluded that PIO at 10 mg/kg may show reduced oxidative stress in heart on isoproterenol induced myocardial infarction in type 2 diabetic rats.

Keywords: Pioglitazone, antioxidant, isoproterenol, Type 2 diabetic

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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]. Type 2 Diabetes Mellitus is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease. 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].
Oxidative stress has been associated with the pathogenesis of chronic diabetic complications including cardiomyopathy. The ability of antioxidants to inhibit these injuries has raised the possibility of newer therapeutic treatment for diabetic heart diseases.

Recently, a protective effect of pioglitazone against oxidative stress in liver and kidney of diabetic rabbits [5] has been reported. Pioglitazone [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 in vitro antioxidant activity in heart of effect of Pioglitazone on isoproterenol induced myocardial infarction in normal and diabetic in rats has not been studied. Hence, the purpose of the present study was to instigate the effect of Pioglitazone treatment on in vitro antioxidant heart tissue parameter alteration in Isoproterenol Induced myocardial infarction in normal and 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*. The animal experiment was approved by Animal Ethical Committee of the Institute (1163/a/08/CPCSEA).

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.

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At the end of experimental period (i.e. on the day 31) 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.

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 (ND-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 biomarkers of Oxidative stress

The excised liver was then weighed and homogenized in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 0oC for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of following antioxidant parameters. The levels of Lipid peroxidation (LPO) formation and the activities of endogenous antioxidant enzymes such as catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) were estimated by the method of Slater and Sawyer [12] Hugo Aebi as given by Hugo [13] Moron et al [14] and Mishra and Fridovich [15].

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).

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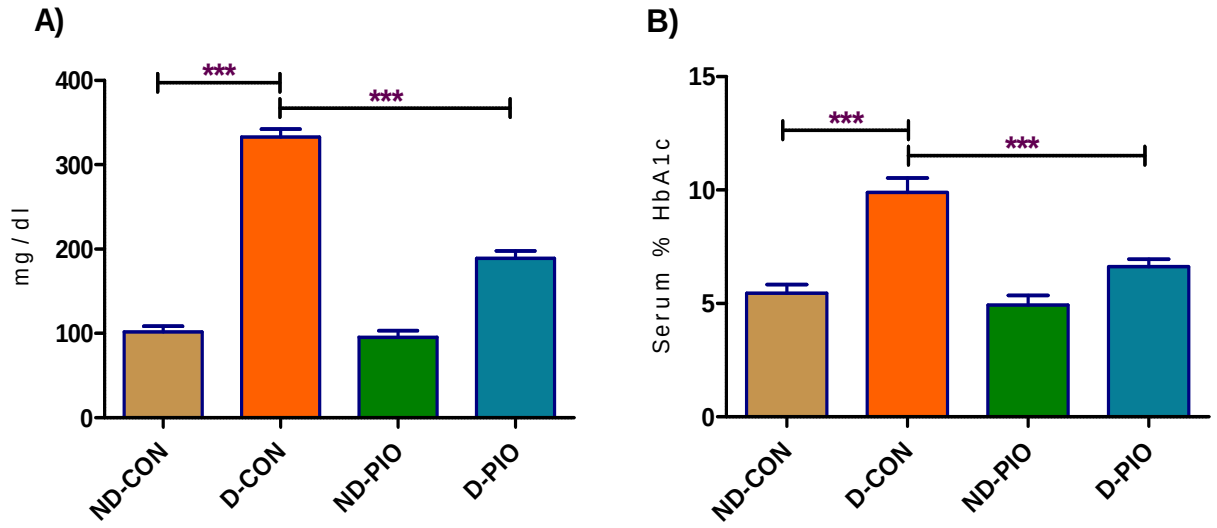
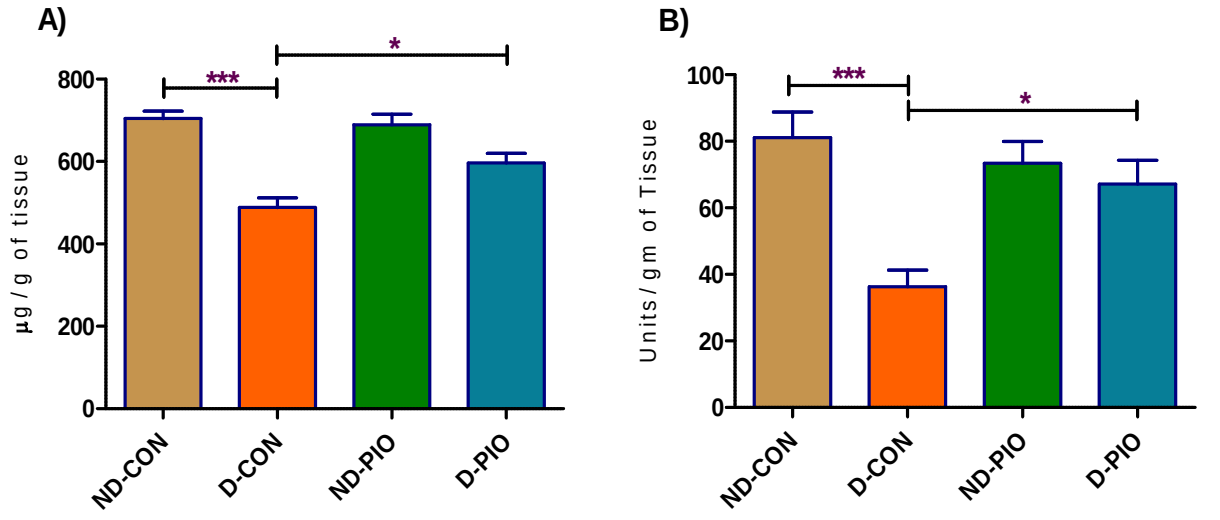
Figure 1. Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.



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.

Figure 2. Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in GSH (A), SOD (B), MDA(C) and CAT (D) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. .



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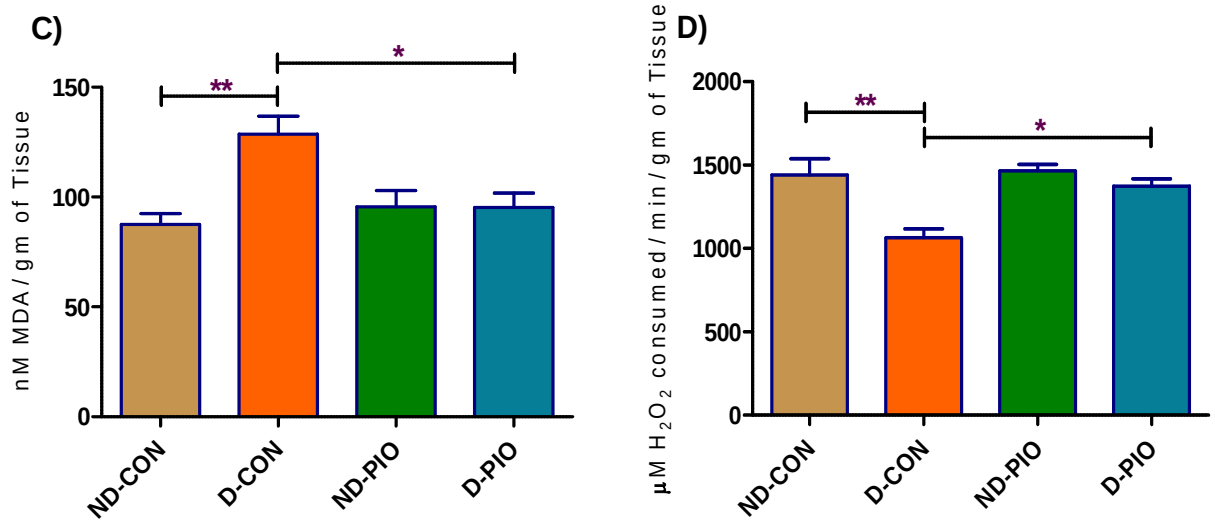

Values are expressed as mean ± SEM for six animals in the group. * P<0.05, ** P<0.001, *** P<0.001 considered statistically significant as compared to respective Control group.

Effect of PIO on myocardial tissue parameter

There was a significantly (P<0.001) decrease in GSH level, (P<0.001) along with SOD and catalase activity (P<0.01) and increased lipid peroxidation (P<0.001) after myocardial infarction in STZ-NIC group (fig. 2). Treatment with Pioglitazone significantly (P<0.05) restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation significantly (P<0.05) in D-PIO group (fig. 2).

DISCUSSION

The present study was under taken with the objective of exploring evaluate in vitro antioxidant activity in heart of Pioglitazone on isoproterenol induced myocardial infarction in normal and diabetic in rats. Recent studies have suggested that prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing. Peroxisome proliferator-activated receptors are nuclear transcription factors that play a role in insulin sensitivity [16].

T2DM is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease (CVD) [17], so that it has been suggested that diabetes may be considered a cardiovascular disease [18]. However, CVD risk is elevated long before the development of diabetes [19].

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 diabetic rats (D-CON) at the end of experimental period. Pioglitazone is reported for its detrimental [20] and protective [21] 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 [22].

Moreover, the levels of endogenous antioxidant (SOD, CAT and GSH) were reduced and lipid peroxidation increased in D-CON group showing increased oxidative stress. Similar results showing increased oxidative stress (increased lipid peroxidation and reduced SOD, CAT and GSH) have been reported in previous studies in STZ

induced diabetes modal [23,24]. PIO at 10 mg/kg may show improve antioxidative stress in heart experimentally induced myocardial infarction in type 2 diabetic rats.

Administration of STZ-NIC caused decrease in SOD, CAT, and GSH but increase in MDA. Treatment with Pioglitazone (10 mg/kg, p.o) could improve result them. This study concluded that PIO at 10 mg/kg may show reduced oxidative stress in heart on isoproterenol induced myocardial infarction in type 2 diabetic rats.

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