HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF PIOGLITAZONE IN NORMAL AND STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

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Summary

Present study was designed to evaluate Hypoglycemic and hypolipidemic activity of pioglitazone in normal and Streptozotocin-Nicotinamide induced 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). Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c), Total Cholesterol (TC), Triglycerides (TG) and High density lipoprotein (HDL) whereas the levels of Low density lipoprotein (LDL)) were found to be non significant. We concluded that PIO (10 mg/kg) is effective in controlling blood glucose levels and improves lipid profile in euglycemic as well as diabetic rats.

Keywords: Pioglitazone, Antioxidant, Hepatotoxicity, Streptozotocin, Nicotinamide

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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). NIDDM has also been associated with an increased risk for premature arteriosclerosis due to increase in triglycerides and low density lipoprotein levels. About 70-80 % of deaths in diabetic patients are due to vascular diseases. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevent the development of arteriosclerosis and other complication of diabetes.

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 (3). Pioglitazone- a PPAR-γ agonist lowers blood pressure and restores blunted endothelium dependent vasodilatation in fructose fed rats, insulin-resistant Rhesus monkey.

Literature survey showed that, there was no report regarding the effect of PIO on glucose, HbA1c and lipid parameter in 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 (4). Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study.

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 (**ND-CON**)].

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**)].

Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (**D-CON**)].

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**)]

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 4^{th} 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, TC, TG, HDL and LDL were estimated using diagnostic kits (SPAN Diagnostics Pvt. India).

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

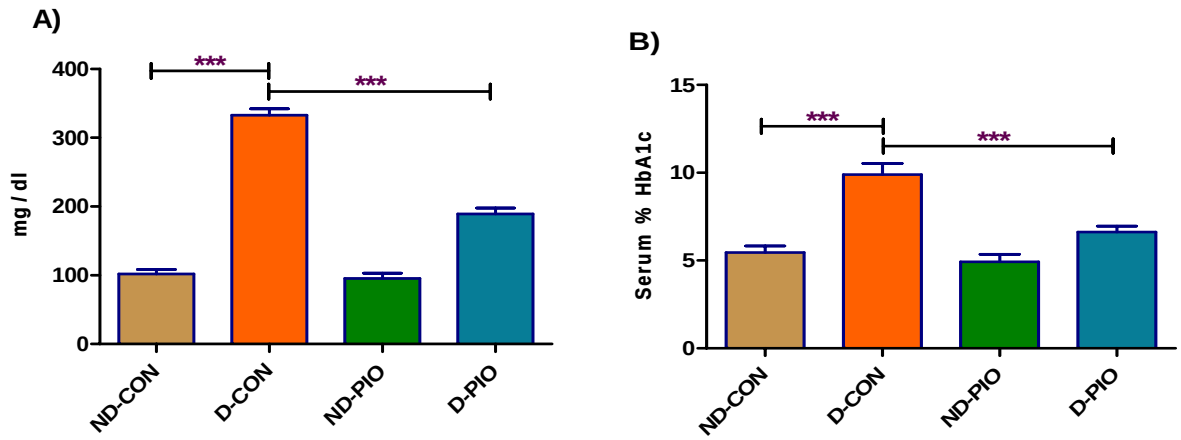
Characterization of Type 2 Diabetes

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.

Effect of PIO on serum enzymes

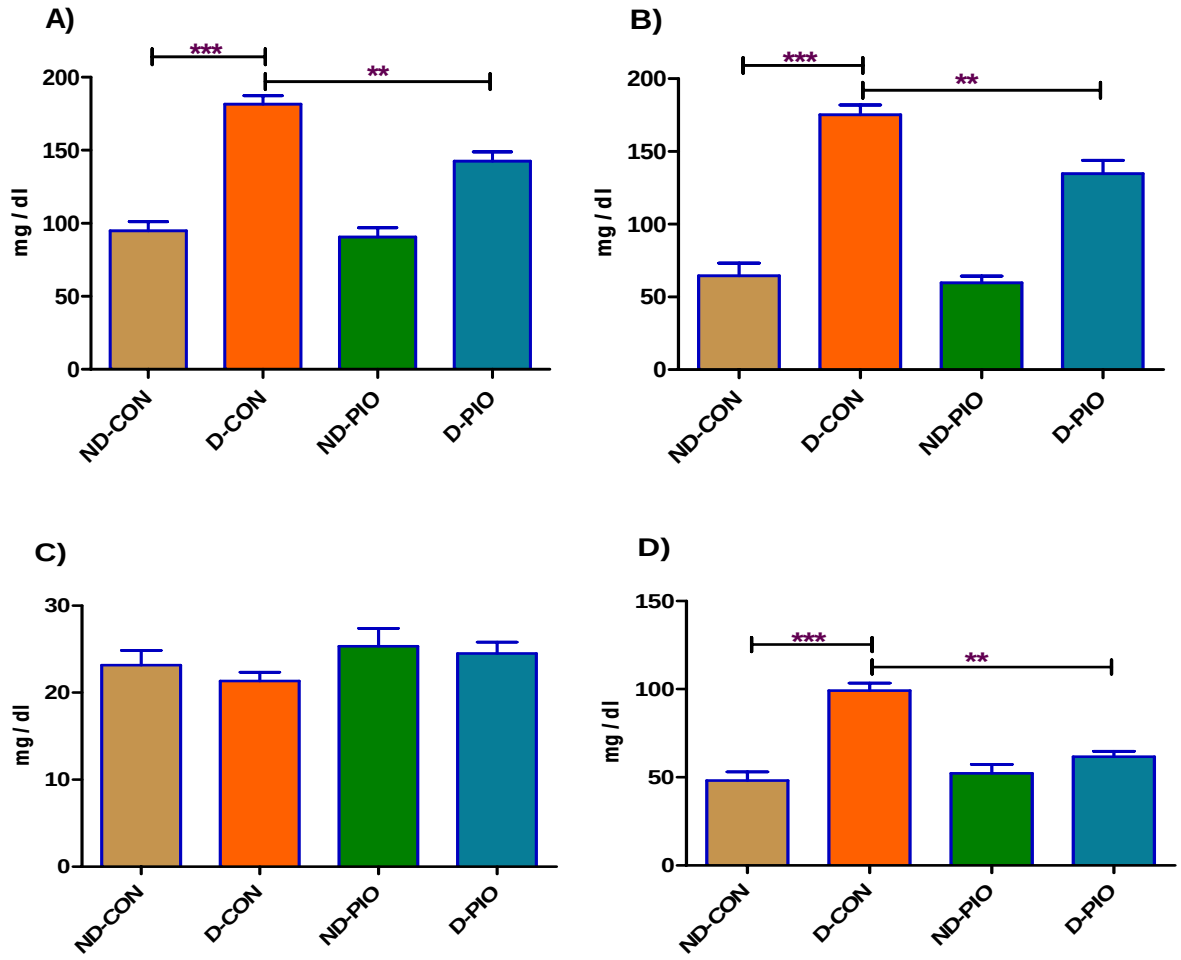
There was a significant ($p<0.001$) increase in blood glucose and HbA1c level of STZ-NIC injected animals compared to ND-CON group (Fig. 1). Serum total cholesterol, LDL and triglyceride were significant ($p<0.001$) increase in STZ-NIC diabetic rats as compared to non-diabetic rats. Administration of PIO in STZ-NIC diabetic rats (D-PIO) significant ($p<0.001$) decrease on blood glucose and HbA1c, while it significantly significant ($p<0.01$) reduced serum cholesterol, triglyceride and LDL ($p< 0.001$) in D-PIO group as compared to D-CON group but there was no significant changes in the levels of HDL (Fig. 2).

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 Control group.

Figure 2. Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in serum Total Cholesterol (A), Triglycerides (B), High density lipoprotein (C) and Low density lipoprotein (D) 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 Control group.

Discussion

The present study was under taken with the objective of exploring the hypoglycemic and hypolipidemic of PIO in STZ-NIC induced 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 (5).

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to chronic complication. Premature and extensive arteriosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occure in diabetes and this is likely to increase the risk for coronary heart disease. A reduction in serum lipids, particularly of the LDL and VLDL fraction and TG, should be considered as being beneficial for the long term prognosis of these patients. Lower of blood glucose and plasma lipid levels thought dietary modification and drug therapy seem to be associated with a decrease in the risk of vascular disease.

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. STZ causes diabetes by the rapid depletion of β-cells and thereby brings about a reduction in insulin release. HbA1c level has been reported to be increased in patients with diabetes mellitus (6). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (7).The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes (8). Elevated levels of HbA1c observed in our study reveal that diabetes animals had prior high blood glucose level. The chronic diabetic state was also associated with dyslipedemia. Administration of STZ caused increase in serum TC, TG, LDL and Pioglitazone (10 mg/kg, p.o) could reduce them. This study concluded that PIO at 10 mg/kg may show some protection on glucose, HbA1c and lipid parameter in STZ-NIC induced diabetic rats. We concluded that PIO (10 mg/kg) is effective in controlling blood glucose levels and improves lipid profile in euglycemic as well as diabetic rats.

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