EFFECT OF GLIMEPIRIDE ON OXIDATIVE STRESS IN HEART – A EXPEROMENTALLY INDUCED MYOCARDIAL INFARCTION IN DIABETIC RATS

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Summary

Present study was designed to evaluate in vitro antioxidant activity in heart of Glimepiride on isoproterenol induced myocardial infarction in normal and diabetic in rats. Glimepiride (0.5 mg/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 (200 mg/kg, s.c.) 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) and nitrite level in heart 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 Glimepiride significantly restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation and nitrite in compared to diabetic control group. This study concluded that GLI at 10 mg/kg may show reduced oxidative stress in heart on isoproterenol induced myocardial infarction in type 2 diabetic rats.

Keywords: Glimepiride, Oxidative Stress, 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.

Glimepiride (GLI) an oral blood glucose lowering drug of the sulfonylurea class is reported to have pancreatic and extra pancreatic effects as well. The blockages of K_{ATP} channels of pancreatic cells by sulphonylurea are critical in the regulation of glucose regulated insulin secretion. It has been demonstrated to be effective in the treatment of non-insulin dependent diabetes mellitus with insulin resistance. GLI is pharmacologically distinct from glibenclamide because of differences in receptor-binding properties which could result in a reduced binding to cardiomyocyte K_{ATP} channels. Some have reported the antioxidative properties of GLI. Moreover, GLI is pharmacologically different than other sulfonylurea drugs for its cardiovascular effects.

So far in vitro antioxidant activity in heart of effect of Glimepiride 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 Glimepiride 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

Glimepiride 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 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 (5).

Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study. Glimepiride (0.5 mg/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) 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 GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-GLI**)] 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 (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 4: STZ-NIC diabetic rats treated with GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-GLI**)] and received ISO (200 mg/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 0 ^{o}C 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 (6) Hugo Aebi as given by Hugo (7) Moron et al (8) and Mishra and Fridovich (9). The clear supernatant was used for estimation of nitrite level (10).

Statistical Analysis

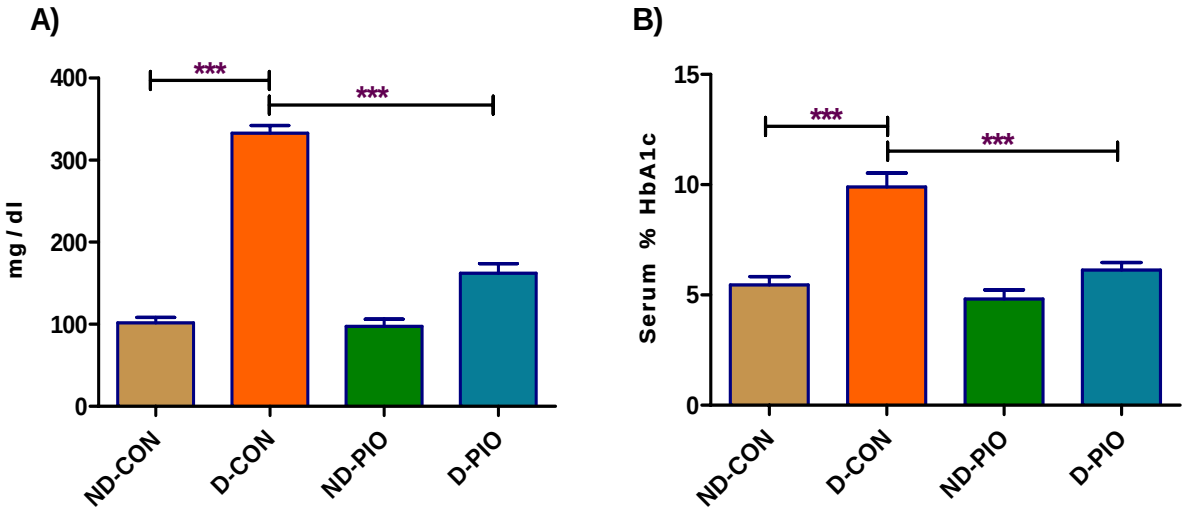
All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences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

Results

Characterization of Type 2 Diabetes

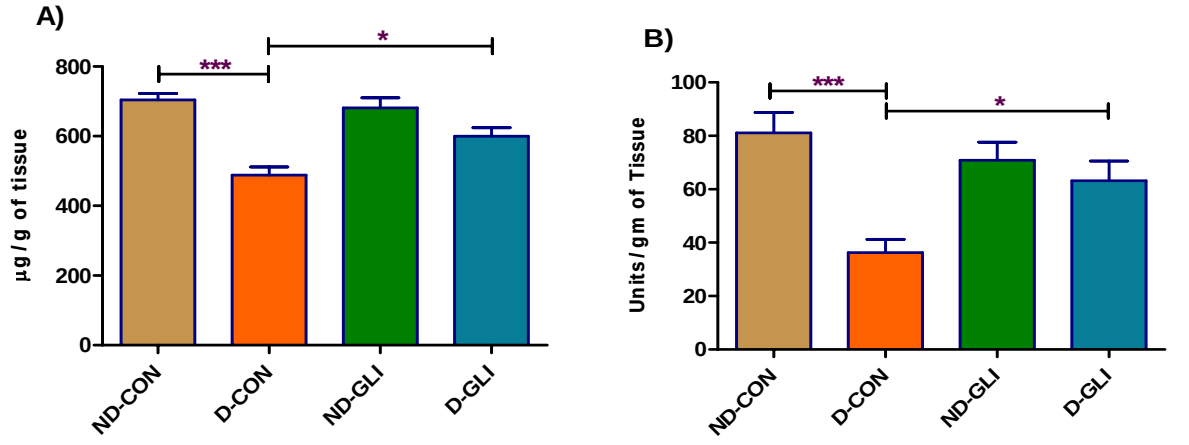
Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Figure 1). The levels of glucose and HbA1c was significant (P<0.001) decreased after treatment with GLI (0.5 mg/kg) alone and combination with GLI (10 mg/kg, p.o) as compared to DB-CON rats.

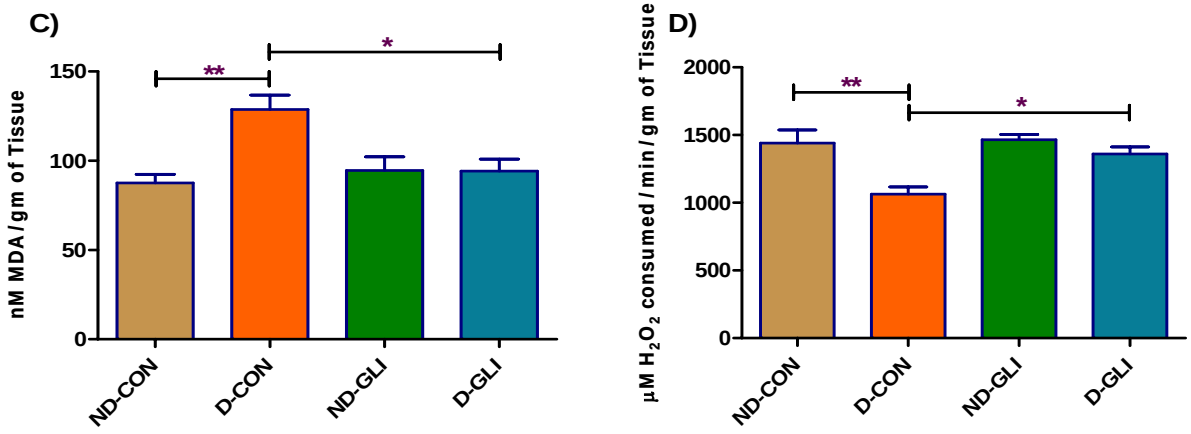
Figure 1. Effect of Glimepiride (0.5 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.



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

Figure 2. Effect of Glimepiride (0.5 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. .



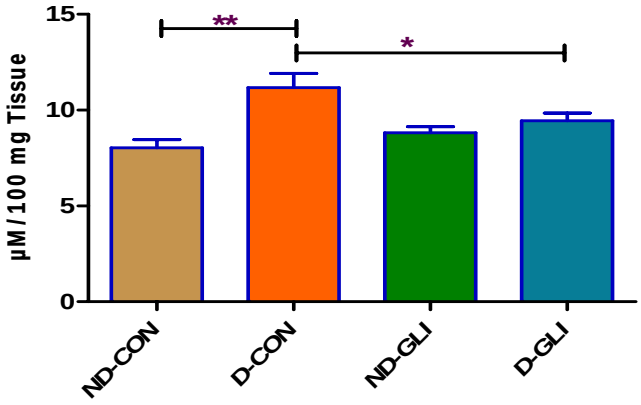


Values are expressed as mean ± SEM for six animals in the group. * P<0.05, **P<0.001, considered statistically significant as compared to respective Control group.

Effect of GLI 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 Glimepiride significantly (P<0.05) restored GSH level, SOD as well as catalase activity and reduced lipid peroxidation significantly (P<0.05) in D-GLI group compared to D-CON group (fig. 2). There was a significant (P < 0.01) increase in cardiac nitrite level in D-CON group as compared to ND-CON group after myocardial infarction. Glimepiride treatment in diabetic rats (D-GLI) significantly (P < 0.05) reduced nitrite level in heart as compared to D-CON group (Fig. 3).

Figure 3. Effect of Glimepiride (0.5 mg/kg/day, p.o) on myocardial changes in Nitrite 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, considered statistically significant as compared to respective Control group.

Discussion

The present study was under taken with the objective of exploring evaluate in vitro antioxidant activity in heart of Glimepiride 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. T2DM is mainly characterized by the development of increased morbidity and mortality for cardiovascular disease (CVD) (11), so that it has been suggested that diabetes may be considered a cardiovascular disease (12). However, CVD risk is elevated long before the development of diabetes (13).

GLI is reported to have extra pancreatic activity which may also involve intensification of trance membrane transport of glucose and an increase in the glycolysis activity.

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 GLI (0.5 mg/kg) when compared with diabetic rats (D-CON) at the end of experimental period.

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 (14). The antioxidant enzymes were restored significantly by GLI treatment in STZ diabetic rats. GLI at 0.5 mg/kg may show improve antioxidative stress in heart experimentally induced myocardial infarction in type 2 diabetic rats.

Myocardial infarction causes further increase in oxidative stress and reduction in nitric oxide availability due to endothelial dysfunction. The destruction of nitric oxide is much prominent in STZ-NIC diabetic rats which is due to increase in ROS formation. Nitric oxide is rapidly inactivated by O_2^- and it has been reported that an enhanced formation of O_2^- radical may be involved in the accelerated break down of nitric oxide (15, 16). GLI treatment reduces the nitrosative stress which is evident from myocardial nitrite level reduction. Thus it may be one of the reasons for the cardioprotective effect of GLI against myocardial infarction.

There may be several mechanisms for cardioprotection by Glimepiride against Myocardial infarction. It may be due to reduction in hyperglycemia induced deleterious effects, reduction in NO destruction, utilization of glucose and restoration of endogenous antioxidants in STZ-NIC diabetic rats.

Administration of STZ-NIC caused decrease in SOD, CAT, and GSH but increase in MDA. Treatment with Glimepiride (0.5 mg/kg, p.o) could improve result them. This study concluded that GLI 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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References

1. Kahn SE, Porte DJ. The pathophysiology of type II (noninsulin-dependent) diabetes mellitus: Implications for treatment. In: Rifkin H, Porte DJ, eds. Ellenberg and Rijkin's Diabetes Mellitus: Theory and Practice. New York: Elsevier Science 1990:436-456.

2. Leibowitz HE. Oral hypoglycemic agents. In: Rifkin H, Porte DJ, eds. Ellenberg nd Rijkin's Diabetes Mellitus: Theory and Practice. New York: Elsevier Science 1990:554-574.

3. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, et al. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res 2001; 88:1291– 8.

4. Senses V, Ozyazgan S, Ince E, Tuncdemir M, Kaya F, Ozturk M, et al. Effect of 5-aminoimidazole-4-carboxamide riboside (AICA-r) on isolated thoracic aorta responses in streptozotocin-diabetic rats. J Basic Clin Physiol Pharmacol 2001; 12:227– 48.

5. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G.. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and Nicotinamide. Diabetes 1998:47, 224–229.

6. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogeno alkanes or peroxidative reactions in rat liver fractions in vitro. Biochem J 1971; 123:805– 14.

7. Hugo EB. Oxidoreductases acting on groups other than CHOH: catalase. In: Colowick SP, Kaplan NO, Packer L, editors. Methods in Enzymology, vol. 105. London 7 Academic Press, 1984; 121– 5.

8. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582:67– 78.

9. Mishra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biochem 1972; 247:3170– 5.

10. Guevara S M, Iwanejko J, Dembinaka-Kiec A, Pankiewicz J, Wanat A, Anna P, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Act 1988; 274: 177-88.

11. Gang Jee Ko, Young Sun Kang, Sang Youb Han, Mi Hwa Lee, Hye Kyoung Song, Kum Hyun Han, Hyoung Kyu Kim, Jee Young Han and Dae Ryong Cha. GLIglitazone attenuates diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. Nephrology Dialysis Transplantation 2008 23(9):2750-2760.

12. Kannel WB, McGee DL. Diabetes and cardiovascular diseases. The Framingham Study. JAMA. 1979; 241:2035-2038.

13. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001; 285:2486-2497.

14. Xu Y, Gen M, Lu L, Fox J, Weiss S O, Brown R D, Perlov D, Ahmad H, Zhu P, Greyson C, Long CS, and Schwartz G G: PPAR-gamma activation fails to provide myocardial protection in ischemia and reperfusion in pigs. Am J Physiol heart circ Physiol 2005; 288: H1314-H1323.

15. Graylewski R J, Palmer R M, Moncada S. Superoxidte anion is involved in the breakdown of endothelium derived vascular relaxing factor. Nature 1986; 320: 454-456.

16. Rubanyi G M, Vanhoutte P M. Superoxide anions and hyperoxia inactive endothelium derived relaxing factor. Am J Physiol 1986; 250: H822-H827.