EFFECT OF GLIMEPIRIDE ALONE AND ITS COMBINATION WITH PIOGLITAZONE AND METFORMIN ON LIVER FUNCTIONS AND BIOMARKER OF OXIDATIVE STRESS IN DIABETIC RATS

Jagdish Kakadiya, S. P. Rathod, R. Balaraman

Pharmacy Department, Faculty of Technology and Engineering, The M.S. University of Baroda, Baroda-390 001, Gujarat, INDIA.

Summary

In the present study effect of Glimepiride (GLI) alone and its combination with Metformin (MET) and Pioglitazone (PIO) was investigated in non diabetic and streptozotocin-nicotinamide induced diabetic and associated hepatic dysfunctioning in rats. Glimepiride(0.5 mg/kg/day, p.o) alone and its combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, 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). STZ–NIC induced animals showed a significant ($p<0.001$) increased in the level of serum glucose, glycosylated heamoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (γGTP). The level of 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. There was no significant changes in the level of total bilirubin (TB) were observed. Treatment with GLI (0.5 mg/kg/day, p.o) alone and in combination Metformin (50 mg/kg/day, p.o) with Pioglitazone (10 mg/kg/day, p.o) showed a significant alteration in all the serum markers and biomarkers of oxidative stress towards normal. Histopathological changes are also in correlation with biochemical alterations. GLI (0.5 mg/kg/day, p.o) alone and in combination Metformin (50 mg/kg/day, p.o) with Pioglitazone (10 mg/kg/day, p.o) found to be effective in protecting STZ–NIC induced Diabetic condition. This study indicates that GLI alone may be better than GLI combination with MET and PIO in protecting hepatic functions in diabetic conditions.

Keywords: Glimepiride, Metformin, Pioglitazone, Antioxidant, Hepatotoxicity, Type 2 diabetic

Address for correspondence
Mr. Jagdish L. Kakadiya
Pharmacy Department, Faculty of Technology and Engineering,
The M.S. University of Baroda, Baroda-39001
jagdishkakadiya@gmail.com

Introduction

Recent epidemiological studies suggested that patients with diabetes are twice as likely to suffer hepatic failure compared to patients who do not have diabetes. Increased incidences of hepatotoxicity have been observed in patients with diabetes receiving drug therapies. Neither the mechanisms nor the predisposing factors underlying hepatotoxicity in patients with diabetes are clearly understood (1). Type 2 diabetes (T2D) is a progressive disorder with a consistent and steady increase in HbA1c over time associated with enhanced risk of micro- and macrovascular complications and a substantial reduction in life expectancy. There are three major pathophysiologic abnormalities associated with T2D: impaired insulin secretion, excessive hepatic glucose output and insulin resistance in skeletal muscle, liver and adipose tissue.

The oxidative stress is thought that also in case of diabetes an increase of reactive oxides and peroxides of lipids occurs along with the lower activity of antioxidative factors (2–4). Mechanism which is responsible for the development of oxidative stress in diabetes has not been univocally determined. A factor probably of greatest significance is hyperglycemia occurring with hypoinsulinemia (5). Normalization of glucose level may thus be a factor inhibiting the development of oxidative stress in diabetes.

Glimepiride is an insulin secretagogue in the Sulfony Urea family and Pioglitazone HCL is an insulin sensitizer in the TZD family. MET improves insulin sensitivity, decreases insulin levels and controls hyperglycemia (6, 7).

Glimepiride has been developed for glycemic control in diabetic patients and represents the third generation sulphonylurea. It effectively inhibits the development of oxidative stress in diabetes (8) by possessing a potent extrapancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway (9).

Metformin improves lipid profiles and lowers blood pressure in both patients and animal models with impaired glucose tolerance and type 2 diabetes mellitus (10-13). MET works in a number of ways to decrease the amount of sugar in the blood. Firstly, it reduces the amount of sugar produced by cells in the liver. Secondly, it increases the sensitivity of muscle cells to insulin. This enables these cells to remove sugar from the blood more effectively.

PIO Hydrochloride is a PPARγ agonist that increases both insulin-stimulated glucose uptake in peripheral tissues (14) and insulin sensitivity in hepatic and adipose tissue (15, 16), there by lowering plasma glucose both as single agent and in combination with other oral hypoglycemic agents and/or insulin (17-20). Pioglitazone has also been shown to have multiple beneficial effects on lipid metabolism (21-25), endothelial function (26, 27), atherogenesis (28, 29), fibrinolysis (30), and immune function (31-33). PIO also reduces the amount of glucose produced by the liver, and preserves the functioning of the cells in the pancreas (beta cells) that produce insulin.

GLI and its combination with MET and PIO is used for people with type 2 diabetes who do not use daily insulin injections. Troglitazone one of the drug

from the PIO class, which was withdrawn from the U. S. market in 2000 because of his high incidence of hepatotoxicity (34).
Literature survey showed that, there was no report regarding the effect of Glimepiride alone and its combination with Metformin and Pioglitazone on the hepatic function diabetic rats. Therefore the above study was designed to evaluate the effect of GLI alone and along with MET and PIO on hepatic functions and biomarkers of oxidative stress in STZ-NIC induced diabetic model in rats.

Materials and method

Drugs and Chemicals

Glimepiride, Pioglitazone HCL and Metformin HCL were obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained form SIGMA, St. Louis, MO, USA. 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 Pharmacy department, The M.S. University of Baroda. 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 overnight fasted rats by a single intraperitoneal injection of 65 mg/kg STZ, 15 min after the i.p administration of 110 mg/kg of NIC (35). After 7 days following STZ and NIC administration, blood was collected from tail vein and serum samples were analyzed for blood glucose. Animals showing fasting blood glucose higher than 300 mg/dl were considered as diabetic and were used for the study.

Experimental Protocol

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

Group 1: Nondiabetic control, received CMC as vehicle (1ml/kg/day, p.o, ND-CON).
Group 2: Nondiabetic group treated with GLI (0.5 mg/kg/day, p.o, ND-GLI).
Group 3: Nondiabetic group treated with GLI (0.5 mg/kg/day, p.o) and it combination with PIO (10 mg/kg/day, p.o) and MET (50 mg/kg/day, p.o, ND-GLI+MET+PIO).
Group 4: Diabetic control, single injection of STZ (65 mg/kg, i.p) and NIC (110 mg/kg, i.p, D-CON).

Group 5: Diabetic rats treated with GLI (0.5 mg/kg/day, D-GLI).
Group 6: Diabetic rats treated with GLI (0.5 mg/kg/day, p.o) and with PIO (10 mg/kg/day, p.o) and MET (50 mg/kg/day, p.o) (D- GLI +MET+ PIO).

Biochemical Estimations

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring no fasting serum glucose (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic (DB) 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} weeks 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, AST, ALT, ALP, γGTP and TB were estimated from serum sample using standard Diagnostic Kit. In vitro quantitative determination of the activity of AST, ALT and TB (SPAN Diagnostics Pvt., India) ALP, γGTP (Crest Biosystems, India) were done using enzymatic kit in serum.

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 (36), Hugo Aebi as given by Hugo (37), Moron et al (38) and Mishra and Fridovich (39).

Histopathology of liver

For light microscopic evaluation, liver tissues of each group were fixed in 10% phosphate buffered formalin. Paraffin-embedded specimens were cut into 6 mm-thick sections and stained with hematoxylin and eosin (H&E). The liver tissues were examined under a light microscope (Olympus Bioxl) for the presence of tubular changes and interstitial inflammatory cell infiltration by an observer blinded to the animal treatment group.

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.

Table 1 showed a significant (P<0.001) decrease in body weight levels in STZ-NIC treated rats (DB-CON) as compared to ND-CON animals. As shown in table1, treatment with GLI (0.5 mg/kg/day, p.o) alone and combination with MET (50 mg/kg, p.o) and PIO (10 mg/kg, p.o) showed a significant (P<0.01) increase in body weight as compared to control non-diabetic (ND) rats and DB-CON rats. Table 1 showed a significant (P<0.001) increase in serum glucose and HbA1c levels in STZ-NIC treated rats (DB-CON) as compared to ND-CON animals. The levels of glucose and HbA1c was significant (P<0.001) decreased after treatment with GLI (0.5 mg/kg/day, p.o) alone and combination with MET (50 mg/kg) alone and PIO (10 mg/kg, p.o) as compared to DB-CON rats.

Table 1. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on changes in Body weight, serum glucose and HbA1c level in non diabetic and STZ-NIC induced diabetic rats.

Group	Body weight (gm)	Glucose (mg/dl)	HbA1c (%)
ND-CON	248.33±5.95	101.0±6.17	5.45±0.37
ND-GLI	245.08±11.12	60.92±7.16$^{\$}$	5.08±0.31
ND-GLI+MET+PIO	269.43±12.87$^{\$\$}$	56.89±5.12$^{\$\$}$	3.53±0.36
D-CON	224.83±8.52$^{\$}$	406.8±6.50$^{\$\$\$}$	11.18±0.52$^{\$\$\$}$
D-GLI	246.08±7.69*	167.8±12.05***	7.10±0.42***
D- GLI+MET+PIO	254.35±9.70**	110.7±7.96***	4.83±0.34***

Values are expressed as mean ± SEM for six animals in the group. $^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, considered statistically significant as compared to ND-CON group.*P<0.05, ** P<0.001, ***P<0.001 considered statistically significant as compared to D-CON group.

Effect of PIO on serum marker enzymes

Figure 1 showed a significant (P<0.001) increase in serum AST and ALT levels in STZ-NIC treated rats (DB-CON) as compared to ND-CON animals. Treatment with GLI (0.5 mg/kg) for 4 weeks, showed further decrease in serum AST and ALT level (P<0.01) as compared to DB-CON group alone. Whereas treatment with GLI (0.5 mg/kg) combination with MET (50 mg/kg) and PIO (10 mg/kg) for 4 weeks showed significant (P<0.05) increase changes in the serum levels AST and ALT level as compared to DB-CON group alone.

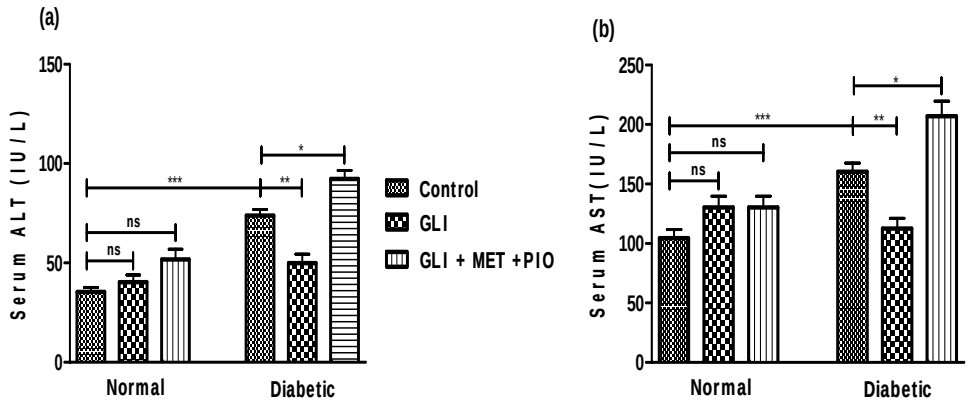

Figure1. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on changes in serum ALT (a) and AST (b) level in non diabetic and STZ-NIC induced diabetic rats. Values are expressed as mean ± SEM for six animals in the group. *P<0.05, **P<0.001, ***P<0.001and ns-no significant considered statistically significant as compared to Control group.

Administration of STZ-NIC alone significantly increases ALP (P<0.001) and γGTP (P<0.05) levels as compared to control rats but there was no significant changes in the levels of TB. As shown in table 2, treatment with GLI (0.5 mg/kg, p.o) showed a significant (P<0.01) decrease in ALP, γGTP and TB as compared to DB control rats. Whereas treatment with GLI (0.5 mg/kg) combination with MET (50 mg/kg) and PIO (10 mg/kg) for 4 weeks showed significantly increases ALP (P<0.05) and γGTP (P<0.05) levels as compared to DB control rats.

Table 2. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on changes in ALP, γGTP and Total bilirubin level in non diabetic and STZ-NIC induced diabetic rats.

Group	ALP (IU/L)	γGTP (IU/L)	TB (IU/L)
ND-CON	138.2± 7.43	76.52± 4.29	0.7192±0.0419
ND-GLI	146.7± 10.57	82.65± 6.46	0.7341±0.0382
ND-GLI+MET+PIO	160.0± 10.73	98.59± 11.14	0.8920±0.0751
D-CON	194.2± 12.22$^{\$\$\$}$	115.9± 7.10$^{\$}$	0.8914±0.0567
D-GLI	133.0± 12.12**	69.66± 8.02**	0.5051±0.0767**
D- GLI+MET+PIO	247.0± 14.62*	157.8± 13.85*	1.062±0.1221

Values are expressed as mean ± SEM for six animals in the group. $^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001 considered statistically significant as compared to ND-CON group; * P<0.05, ** P<0.001, ***P<0.001 considered statistically significant as compared to D-CON group.

Effect of PIO on Biomarkers of oxidative stress

MDA level was significantly (p<0.001) increased and the levels of GSH, CAT and SOD were significantly (p<0.001) decreased in STZ-NIC treated rats when compared with those of the animals in control group. Treatment with GLI (0.5 mg/kg) showed significantly (p<0.01) decreased MDA and increased the levels of GSH (p<0.01), CAT (p<0.01) and SOD (p<0.01) (Fig. 2). Whereas treatment with GLI (0.5 mg/kg) combination with MET (50 mg/kg) and PIO (10 mg/kg) for 4 weeks, showed no significantly MDA and no significant the levels of SOD, CAT and significantly (p<0.05) decreased GSH changes in the tissue levels as compared to DB-CON group and NB-CON group.

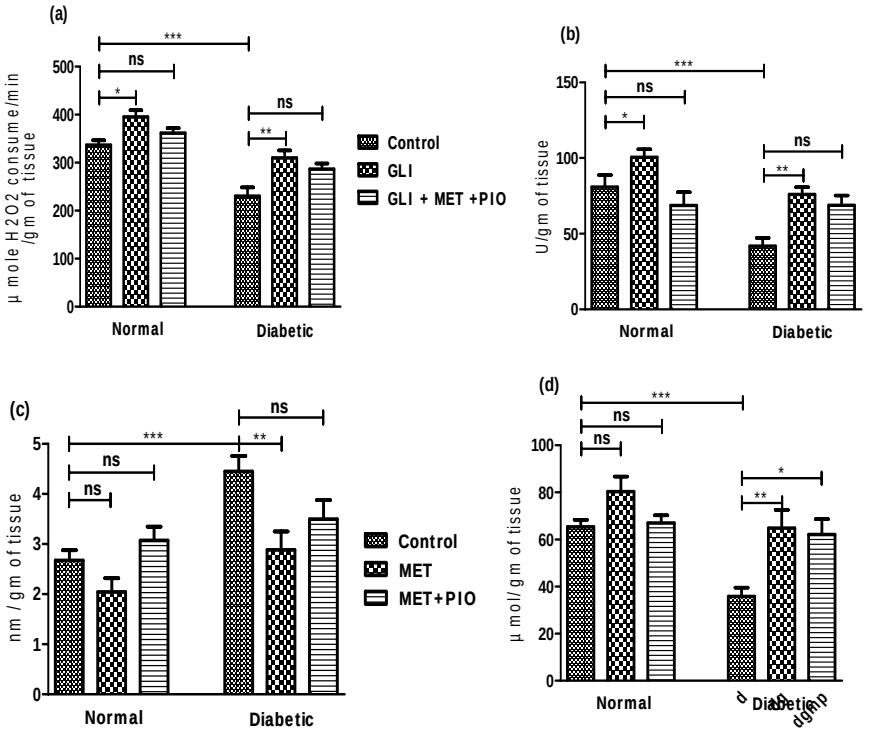

Figure 2. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on CAT (a), SOD (b), MDA (c) and GSH (d) level in non diabetic and STZ-NIC induced diabetic rats. (a) Catalase (CAT), b) Superoxide dismutase (SOD), c) lipid peroxidation or malondialdehyde (MDA) and d) reduced glutathione (GSH) levels in rats subjected to after 4 weeks, Values are expressed as mean ± SEM for six animals in the group. *P<0.05, **P<0.001; ***P<0.001 and ns-no significant considered statistically significant as compared to Control group.

Effect of PIO on Histopathological changes

Liver sections were examined by light microscopy (Fig. 3) for necrosis and inflammatory cell infiltration. Livers from untreated ND-CON and DB-CON rats were indistinguishable indicating that diabetes alone has no discernible effects in the liver. In the liver of ND-CON group, rats at 4 weeks after GLI (0.5 mg/kg) alone and alone with MET (50 mg/kg) and PIO (10 mg/kg) administration showed swollen hepatocytes with inflammatory cell infiltration in centrilobular area were minimally evident. In contrast, in the liver of DB rats at 4 weeks after GLI (0.5 mg/kg) administration showed swollen hepatocytes with inflammatory cell infiltration in centrilobular area was minimally evident. Vacuolar degeneration and centrilobular necrosis of hepatocytes and mild inflammation were observed at 4 weeks after GLI (0.5 mg/kg) and combination with MET (50 mg/kg) and PIO (10 mg/kg) administration.

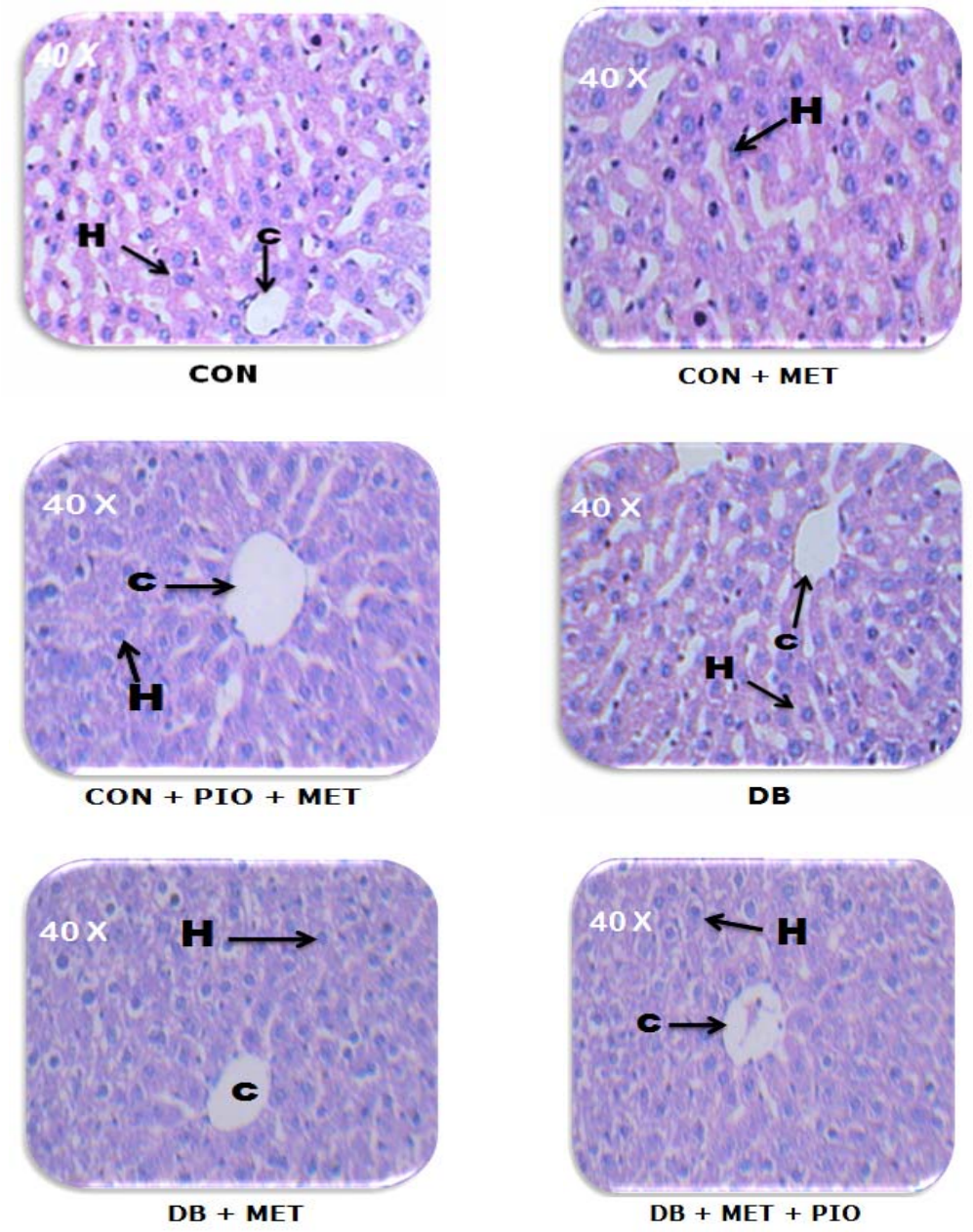

Figure 3. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) and Pioglitazone (10 mg/kg/day, p.o) on Liver tissues in non diabetic and STZ-NIC induced diabetic rats. **C** = Central vein, **N** = Hepatocytes Necrosis, **H** = Normal Hepatocytes

Discussion

The present study was undertaken with the objective of exploring the hepatic function of GLI alone and its combination with MET and PIO in STZ-NIC induced diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing.

In STZ-NIC induced diabetes, the characteristic loss of body weight caused by an increase in muscle wasting (40). In the present study treatment with GLI alone and combination with MET and PIO showed significant increase in body weight which may be because of formation of oedema in the tissue. 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 alone and GLI combination with MET and PIO when compared with DB-CON rats at the end of experimental period. STZ causes diabetes by the rapid depletion of β-cells and thereby brings about an eduction in insulin release. HbA1c level has been reported to be increased in patients with diabetes mellitus (41). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (42).The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes (43).Elevated levels of HbA1c observed in our study reveal that diabetes animals had prior high blood glucose level.

In STZ induced animals a change in the serum enzymes is directly related to changes in the metabolic functions of AST, ALT, ALP and γ- GTP (44-46). It has been reported that the increased levels of transaminases under insulin deficiency (47) were responsible for the increased gluconeogenesis and ketogenesis during diabetes. The increased levels of serum AST, ALT and ALP have already been reported to be associated to liver dysfunction and leakage of these enzymes to the liver cytosol in to the blood stream in diabetes (48). Decreased in the activity of AST, ALT, ALP and γ- GTP in GLI and combination with MET and PIO treated diabetic rats indicate the protective role of the GLI combination with MET and PIO against STZ–NIC induced hepatocellular necrotic changes.

Oxidative stress originating from improper control of the reduction of O_2 is believed to play a role in the tissue and cellular damage caused by a variety of conditions in diabetes (49).The effects of thiazolidinediones on oxidative stress are difficult to predict (50). Previous studies have proved that, thiazolidinedione exposure increase oxidative stress (51). SOD and CAT are considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species (52). SOD is an important defense enzyme, which catalyzes the dismutation of superoxide radicals (53) and CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (54). The reduced activity of SOD and CAT in the liver observed in diabetes may pose deleterious effects as the result of the accumulation of superoxide anion

radicals and hydrogen peroxide (55). GSH, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxidase (56, 57). In our study, the activity of endogenous antioxidants was significantly changed with GLI alone and combination with MET and PIO. Treatment with GLI alone and GLI with MET and PIO further increases the levels of endogenous antioxidants and decreases the level of lipid peroxidation.

This study concluded that GLI alone and combination with PIO and MET may show some protection in STZ-NIC induced diabetic rats whereas with doses and chronic treatment it showed further liver protection but GLI alone may be better than GLI combination with MET and PIO in protecting hepatic functions in diabetic conditions.

References

1. Mary Vagula, Sachin S. Devi. Hepatotoxicity of Antidiabetic Drugs. US Pharm. 2008; 33(5) 3-9.
2. Orrenius S, Mc Conkey DJ, Nicotera P: Mechanisms of oxidant induced cell damage. In: Cerruti PC, Fridovich J, Mc Lord TCM (eds.): Oxy – radicals in molecular biology and pathology. Alan R Liss Inc. Nowy Jork, 1988, 327-339
3. Orrenius S, Mc Conkey DJ, Bellomo G et al: Role of Ca2+ in toxic cell killing. Trends Pharmacol Sci, 1989; 10: 281-85
4. Beals CC, Bullock J, Jauregui ER, Duran WN: Microvascular clearance of macromolecules in skeletal muscle of spontaneously diabetic rats. Microvasc Res, 1993; 45: 11-19
5. Ditzel J: Functional microangiopathy in diabetes mellitus. Diabetes, 1968; 17: 388-97
6. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI. Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. N Engl J Med. 1998; 338:867– 872.
7. Fanghanel G, Sanchez-Reys L, Trujillo C, Sotres D, Espinosa-Campos J. Metformin's effects on glucose and lipid metabolism in patients with secondary failure to sulfonylureas. Diabetes Care. 1996; 19: 1185–1189
8. Kissane, J.M., 1985. Anderson's Pathology, 8th Edn. Toronta: Washington Univ. School Med., pp: 54-759.
9. Takada, Y., Y. Takata, M. Iwanishi, T. Imamura, T. Sawa, H. Morioka, H. Ishihara, M. Ishiki, I. Usui, R. Temaru, M. Urakaze, Y. Satoh, T. Inami, S. Tsuds and M. Kobayashi, 1996. Effect of glimepiride (HOE 490) on insulin receptors of skeletal muscles from genetically diabetic KK-Ay mouse. Eur. J. Pharmacol., 308: 205-210.

10. Fanghanel G, Sanchez-Reys L, Trujillo C, Sotres D, Espinosa-Campos J. Metformin's effects on glucose and lipid metabolism in patients with secondary failure to sulfonylureas. Diabetes Care. 1996; 19: 1185–1189.
11. Sundaresan P, Lykos D, Daher A, Diamond T, Morris R, Howes LG. Comparative effects of glibenclamide and metformin on ambulatory blood pressure and cardiovascular reactivity in NIDDM. Diabetes Care. 1997; 20:692– 697.
12. Bhalla RC, Toth KF, Tan E, Bhatty RA, Mathias E, Sharma RV. Vascular effects of metformin: possible mechanisms for its antihypertensive action in the spontaneously hypertensive rat. Am J Hypertens. 1996; 9:570 –576.
13. Verma S, Bhanot S, McNeill JH. Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J Pharmacol Exp Ther. 1994; 271:1334 –1337.
14. Miyazaki Y, Mahankali A, Matsuda M, et al. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. Diabetes Care. 2001;24:710–9
15. Kawamori R, Matsuhisa M, Kinoshita J, et al. Pioglitazone enhances splanchnic glucose uptake as well as peripheral glucose uptake in non-insulin-dependent diabetes mellitus. AD-4833 Clamp-OGL Study Group. Diabetes Res Clin Pract. 1998; 41:35–43.
16. Miyazaki Y, Mahankali A, Wajcberg E, et al. Effect of pioglitazone on circulating adipocytokine levels and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab. 2004;89:4312–9
17. Aronoff S, Rosenblatt S, Braithwaite S, et al. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. Diabetes Care. 2000; 23:1605–11.
18. Rosenblatt S, Miskin B, Glazer NB, et al. The impact of pioglitazone on glycemic control and atherogenic dyslipidemia in patients with type 2 diabetes mellitus. Coron Artery Dis. 2001;12:413–23
19. Scherbaum WA, Goke B. Metabolic efficacy and safety of once-daily pioglitazone monotherapy in patients with type 2 diabetes: a double-blind, placebo-controlled study. Horm Metab Res. 2002; 34:589–95.
20. Herz M, Johns D, Reviriego J, et al. A randomized, double-blind, placebo-controlled, clinical trial of the effects of pioglitazone on glycemic control and dyslipidemia in oral antihyperglycemic medication-naive patients with type 2 diabetes mellitus. Clin Ther. 2003; 25:1074–95.
21. Winkler K, Konrad T, Fullert S, et al. Pioglitazone reduces atherogenic dense LDL particles in nondiabetic patients with arterial hypertension: a double-blind, placebo-controlled study. Diabetes Care. 2003; 26:2588–94.
22. Khan M, Xu Y, Edwards G, et al. Effects of pioglitazone on the components of diabetic dyslipidaemia: results of double-blind, multicentre, randomised studies. Int J Clin Pract. 2004; 58:907–12.

23. Perez A, Khan M, Johnson T, et al. Pioglitazone plus a sulphonylurea or metformin is associated with increased lipoprotein particle size in patients with type 2 diabetes. Diab Vasc Dis Res. 2004; 1:44–50.
24. Betteridge DJ, Verges B. Long-term effects on lipids and lipoproteins of pioglitazone versus gliclazide addition to metformin and pioglitazone versus metformin addition to sulphonylurea in the treatment of type 2 diabetes. Diabetologia. 2005; 48:2477–81.
25. Goldberg RB, Kendall DM, Deeg MA, et al. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care. 2005; 28:1547–54.
26. Forst T, Lubben G, Hohberg C, et al. Influence of glucose control and improvement of insulin resistance on microvascular blood flow and endothelial function in patients with diabetes mellitus type 2. Microcirculation. 2005b; 12:543–50.
27. Martens FM, Visseren FL, de Koning EJ, et al. Short-term pioglitazone treatment improves vascular function irrespective of metabolic changes in patients with type 2 diabetes. J Cardiovasc Pharmacol. 2005; 46:773–8.
28. Langenfeld MR, Forst T, Hohberg C, et al. Pioglitazone decreases carotid intima-media thickness independently of glycemic control in patients with type 2 diabetes mellitus: results from a controlled randomized study. Circulation. 2005; 111:2525–31.
29. Mazzone T, Meyer PM, Feinstein S, et al. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. JAMA. 2006; 296:2572–81.
30. Derosa G, Cicero AF, D'Angelo A, et al. Effects of 1 year of treatment with pioglitazone or rosiglitazone added to glimepiride on lipoprotein a. and homocysteine concentrations in patients with type 2 diabetes mellitus and metabolic syndrome: a multicenter, randomized, double-blind, controlled clinical trial. Clin Ther. 2006; 28:679–88.
31. Marx N, Mach F, Sauty A, et al. Peroxisome proliferator-activated receptor-gamma activators inhibit IFN-gamma-induced expression of the T cell-active CXC chemokines IP-10, Mig, and I-TAC in human endothelial cells. J Immunol. 2000; 164:6503–8.
32. Gosset P, Charbonnier AS, Delerive P, et al. Peroxisome proliferator-activated receptor gamma activators affect the maturation of human monocyte-derived dendritic cells. Eur J Immunol. 2001; 31:2857–65.
33. Martens FM, Rabelink TJ, op't Roodt J, et al. TNF-alpha induces endothelial dysfunction in diabetic adults, an effect reversible by the PPAR-gamma agonist pioglitazone. Eur Heart J. 2006; 27:1605–9.
34. Baughman T. M, Graham R. A, Wells-Knecht K, Silver I. S, Tyler L. O, Metabolic activation of pioglitazone identified from rat and human liver microsomes and freshly isolated hepatocytes: Drug metabolism and disposition 2005:733-738.
35. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G., 1998. Experimental NIDDM:

development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 47, 224–229.

36. 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.

37. 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.

38. 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.

39. 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.

40. Swanston-Flat SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes: studies in normal and streptozotocin diabetic mice. Diabetologia 1990; 33:462-464.

41. Paulsen, E.P. Heamoglobin A1C in childhood of diabetes. Metabolism 1973; 22: 269- 271.

42. Koening, R.L., Peterson, C.M. Jones, R.L. Saudek, C. Lehrman, M. and Cerami, A. Correlation of glucose regulation and hemoglobin A1C in diabetes mellitus. New England Journal of Medicine 1976; 295: 417-420.

43. Gabbay, K.H. Glycosylated hemoglobin and diabetic control. New England Journal Medicine 1976; 95: 443-454.

44. Junad A, Lambert AE, Orci L, Pictet R, Gonet AE, Ronald AE. Studies of diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. 1967; 126: 201-205.

45. Efe B, Basaran A, Vardereli E, Kıraç S, Dinçer S, Harmancı A, Eren Z, Erenoglu E. Diabetes mellitus'ta aminositler. Endokrinolojide Yönelisler 1992; 5: 36-43.

46. Asayama K, Nakane T, Uchida N, Hayashihe H, Dobashi K, Nakazawa S. Serum antioxidant status in streptozotocin-induced Diabetic Rat. Horm. Metab. Res. 1994; 26: 313-315.

47. Fleig. P., Marliss, E. Ohman, J. and Cahill Jr, J.F. Plasma amino acid levels in diabetic keto acidosis. Diabetes 1970; 19: 727- 729.

48. Ohaeri, O.C. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Bioscience Reproduction 2001; 21: 19–24.

49. Betty A. Maddux, Wendy See, John C. Lawrence, Jr., Amy L. Goldfine, Ira D. Goldfine, and Joseph L. Evans. Protection Against Oxidative Stress—Induced Insulin Resistance in Rat L6 Muscle Cells by Micromolar Concentrations of □-Lipoic Acid. Diabetes 2001; 50:404-410.

50. Fiskum G, Rosenthal RE, Vereczki V, Martin E, Hoffman GE, Chinopoulos C, and Kowaltowski A .Protection against ischemic brain injury by inhibition of mitochondrial oxidative stress. J Bioenerg Biomembr 2004 ; 36: 347–352
51. Shishido S, Koga H, Harada M, Kumemura H, Hanada S, Taniguchi E, Kumashiro R, Ohira H, Sato Y, Namba M, et al. Hydrogen peroxide overproduction in megamitochondria of troglitazone-treated human hepatocytes. Hepatology 2003; 37: 136–147.
52. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 1985; p. 1-27.
53. McCord JM, Keele BB, Fridovich I. An enzyme based theory of obligate anaerobiosis, the physiological functions of superoxide dismutase. Proc Natl Acad Sci USA 1976; 68:1024-1027.
54. Chance B, Greenstein DS, Roughton RJW. The mechanism of catalase action – steady state analysis. Arch Biochem Biophys 1952; 37:301-339.
55. Searle AJ, Wilson R. Glutathione peroxide effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. Int J Radiat Biol 1980; 37:213-217.
56. Meister A. New aspects of glutathione biochemistry and transport selective alterations of Glutathione metabolism. Nutr Rev 1984; 42:397- 410.
57. Nicotera P, Orrenius S. Role of thiols in protection against biological reactive intermediates. Adv Exp Med Biol 1986; 97:41-49.