PROTECTIVE EFFECT OF GLIMEPIRIDE ALONE AND ITS COMBINATION WITH METFORMIN ON SERUM GLUCOSE, HbA1c AND LIVER FUNCTIONS IN NONDIABETIC AND DIABETIC RATS

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Summary

In the present study effect of Glimepiride (GLI) alone and its combination with Metformin (MET) 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) 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) showed a significant alteration in all the serum markers and biomarkers of oxidative stress towards normal. This study indicates that GLI alone may be better than GLI combination with MET in protecting hepatic functions in diabetic conditions.

Keywords: Glimepiride, Metformin, Streptozotocin, Nicotinamide

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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 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.

GLI and its combination with MET is used for people with type 2 diabetes who do not use daily insulin injections.

Literature survey showed that, there was no report regarding the effect of Glimepiride alone and its combination with Metformin on the hepatic function diabetic rats. Therefore the above study was designed to evaluate the effect of GLI alone and along with MET on hepatic functions and biomarkers of oxidative stress in STZ-NIC induced diabetic model in rats.

Materials and Method

Drugs and Chemicals

Glimepiride 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 (14). 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 its combination with MET (50 mg/kg/day, p.o, ND-GLI+MET).

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) with MET (50 mg/kg/day, p.o) (DB-GLI +MET).

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 (15), Hugo Aebi as given by Hugo (16), Moron et al (17) and Mishra and Fridovich (18).

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) showed a significant (P<0.05) 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 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) 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	257.43±11.25$^{\$}$	59.46±6.34$^{\$}$	3.53±0.36
DB-CON	224.83±8.52$^{\$}$	406.8±6.50$^{\$\$\$}$	11.18±0.52$^{\$\$\$}$
DB-GLI	246.08±7.69*	167.8±12.05***	7.10±0.42***
DB-GLI+MET	247.92±8.83*	132.65±9.46***	5.99±0.26***

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 GLI and MET 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) for 4 weeks showed no changes in the serum levels AST and ALT level as compared to DB-CON group alone.

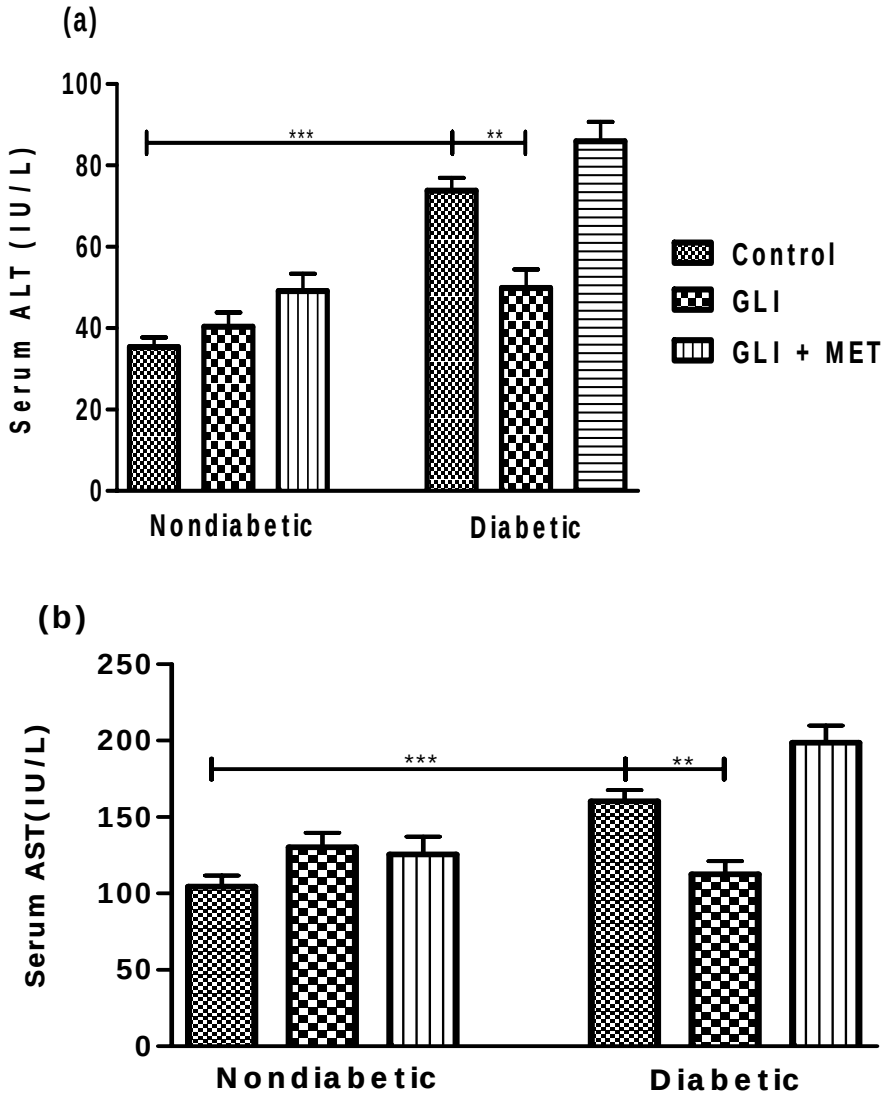

Figure1. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) on changes in serum ALT (a) and AST (b) level in non diabetic and STZ-NIC induced diabetic rats. Values are expressed as mean ± SEM for six animals in the group. $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P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

Table 2. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 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	156.30± 9.33	93.92± 10.4	0.8820±0.0651
DB-CON	194.2± 12.22$^{\$\$\$}$	115.9± 7.10$^{\$}$	0.8914±0.0567
DB-GLI	133.0± 12.12**	69.66± 8.02**	0.5051±0.0767**
DB-GLI+MET	221.30± 12.32*	121.76±9.22	1.000±0.1011

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.

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) for 4 weeks showed significantly increases ALP (P<0.05) levels as compared to DB control rats.

Effect of GLI and MET 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) (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.

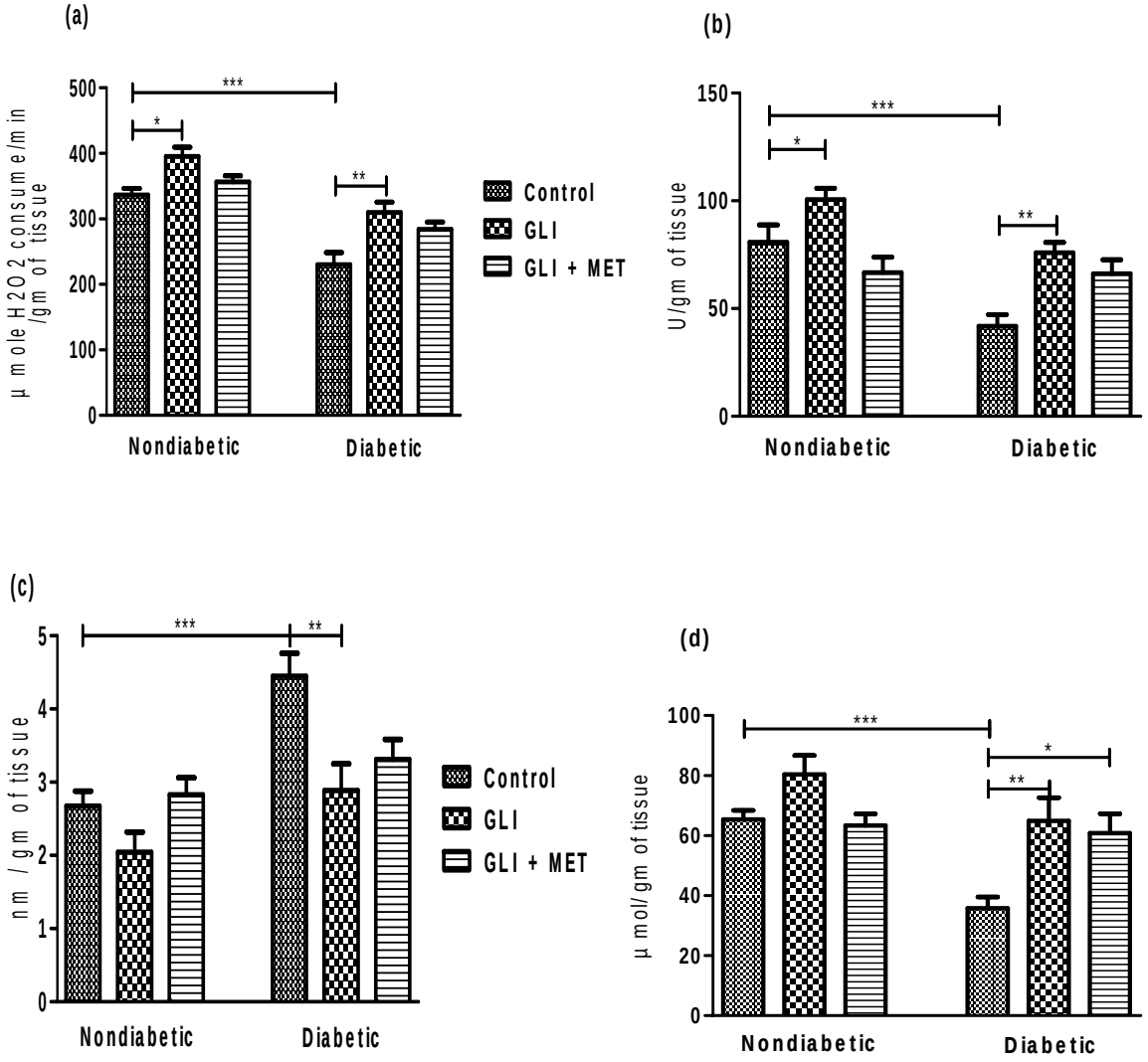

Figure 2. Effect of GLI (0.5 mg/kg/day, p.o) alone and combination with Metformin (50 mg/kg/day, p.o) on CAT (a), SOD (b), MDA (c) and GSH (d) level in non diabetic and STZ-NIC induced diabetic rats. (a) Catalase (CAT), b) Superoxide dismutase (SOD), c) lipid peroxidation or malondialdehyde (MDA) and 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.

Discussion

The present study was undertaken with the objective of exploring the hepatic function of GLI alone and its combination with MET 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 (19). In the present study treatment with GLI alone and combination with MET 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 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 (20). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (21).The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes (22).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 (23-25). It has been reported that the increased levels of transaminases under insulin deficiency (26) 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 (27). Decreased in the activity of AST, ALT, ALP and γ- GTP in GLI and combination with MET treated diabetic rats indicate the protective role of the GLI combination with MET 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 (28).The effects of thiazolidinediones on oxidative stress are difficult to predict (29). Previous studies have proved that, thiazolidinedione exposure increase oxidative stress (30). SOD and CAT are considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species (31).

SOD is an important defense enzyme, which catalyzes the dismutation of superoxide radicals (32) and CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (33). 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 (34). 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 (35, 36). In our study, the activity of endogenous antioxidants was significantly changed with GLI alone and combination with MET. Treatment with GLI alone and GLI with MET further increases the levels of endogenous antioxidants and decreases the level of lipid peroxidation.

This study concluded that GLI alone and combination with 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 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. 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.

15. 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.

16. 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.

17. 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.

18. 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.

19. 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.

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

21. 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.

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

23. 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.

24. 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.

25. 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.

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

27. 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.

28. 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.

29. 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

30. 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.

31. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 1985; p. 1-27.

32. 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.

33. Chance B, Greenstein DS, Roughton RJW. The mechanism of catalase action – steady state analysis. Arch Biochem Biophys 1952; 37:301-339.

34. 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.

35. Meister A. New aspects of glutathione biochemistry and transport selective alterations of Glutathione metabolism. Nutr Rev 1984; 42:397- 410.

36. Nicotera P, Orrenius S. Role of thiols in protection against biological reactive intermediates. Adv Exp Med Biol 1986; 97:41-49.