EFFECT OF METFORMIN ALONE AND ALONG WITH PIOGLITAZONE ON HEPATIC FUNCTION IN DIABETIC RATS

Jagdish Kakadiya, Rajesh Maheshwari, Jitendra Parmar, Aman Upaganlawar, 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 Metformin (MET) alone and along with Pioglitazone (PIO) was investigated in streptozotocin- nicotinamide induced diabetic and associated hepatic dysfunctioning in rats. Metformin (50 mg/kg/day, p.o) alone and its combination with 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. No significant changes were found in the level of total bilirubin (TB). Treatment with Metformin (50 mg/kg/day, p.o) alone and in combination 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. MET (50 mg/kg) alone and its combination with PIO was found to be effective in protecting STZ–NIC induced diabetic condition. This study indicates that MET alone may be better than MET and PIO with combination in protecting hepatic functions in diabetic conditions.

***Key words*:** Metformin, Pioglitazone, Antioxidant, Hepatotoxicity,

Address for correspondence
Mr. Jagdish L. Kakadiya
Pharmacy Department, Faculty of Technology and Engineering,
The M.S. University of 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 glycosylated hemoglobin (HbA_1c) over time associated with enhanced risk of micro-complication (retinopathy, nephropathy and neuropathy) and macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease) and a substantial reduction in life expectancy. Liver disease complication is one of the most common causes of morbidity and mortality in diabetic patients [2].

MET improves insulin sensitivity, decreases insulin levels, and controls hyperglycemia [3, 4]. In addition, metformin improves lipid profiles and lowers blood pressure in both patients and animal models with impaired glucose tolerance and type 2 diabetes mellitus [5-8]. 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 is a type of antidiabetic medicine known as a thiazolidinedione or glitazone. It helps to control blood sugar levels by increasing the sensitivity of liver, fat and muscle cells to insulin. This enables these cells to remove sugar from the blood more effectively. 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.

MET and PIO is used for people with type 2 diabetes who do not use daily insulin injections. 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 lowers blood pressure and restores endothelial function in animals [9]. 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 [10].

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

Materials and Method

Drugs and Chemicals

Pioglitazone hydrochloride and Metformin hydrochloride was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained form 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 Pharmacy department, The M.S. University, 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*.

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 [11]. 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 MET (50 mg/kg/day, p.o, ND-MET).

Group 3: Nondiabetic group treated with PIO (10 mg/kg/day, p.o) and MET (50 mg/kg/day, p.o, ND-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 MET (50 mg/kg/day, D-MET).

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

Biochemical Estimations

Confirmation of diabetes

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 [12] Hugo Aebi as given by Hugo [13] Moron et al [14] and Mishra and Fridovich [15].

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 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

Confirmation of diabetes

As shown in table1, treatment with MET (50 mg/kg) and combination with 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. Administration of STZ-NIC alone (DB-CON) significantly (P<0.001) increases glucose and HbA1c levels as compared to control rats. The levels of glucose and HbA1c was significant (P<0.001) decreased after treatment with MET (50 mg/kg) alone and combination with PIO (10 mg/kg, p.o) as compared to DB-CON rats.

Table 1. Effect of 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 diabetic rats.

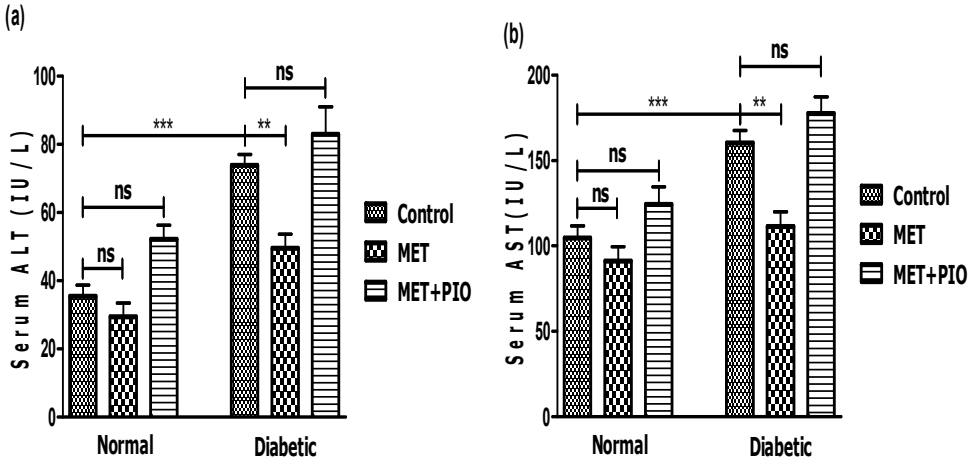
Group	Body weight (gm)	Glucose (mg/dl)	Glycosylated heamoglobin (%, HbA1c)
ND-CON	248.3± 11.86	99.32±7.34	5.48±0.37
ND-MET	250.0± 9.245	79.59±7.06	5.10±0.33
ND-MET+PIO	315.2± 10.55$^{\$\$}$	65.42±8.68	4.16±0.22
D-CON	208.3± 8.92	405.1±9.55$^{\$\$\$}$	11.07±0.55$^{\$\$\$}$
D-MET	221.8±11.68	186.7±12.01***	7.74±0.29***
D- MET+PIO	265.50±8.94**	117.8±10.98***	5.75±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 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 MET (50 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 MET (50 mg/kg) combination with PIO (10 mg/kg) for 4 weeks showed no significant changes in the serum levels AST and ALT level as compared to DB-CON group alone.

Figure 1. Effect of Metformin and Pioglitazone on serum ALT (a) and AST (b) level in non diabetic and 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.

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

Table 2. Effect of Metformin and Pioglitazone on changes in ALP, γGTP and Total bilirubin level in non diabetic and diabetic rats.

Group	ALP (IU/L)	γGTP (IU/L)	TB (IU/L)
ND-CON	134.9±7.08	74.91±4.15	0.7158±0.042
ND-MET	119.8±12.88	75.14±5.49	0.4805±0.046
ND-PIO+MET	147.3±8.69	108.0±8.47$^{\$}$	0.6910±0.044
D-CON	194.3±9.11$^{\$\$\$}$	107.7±4.05$^{\$\$}$	0.8914±0.056
D-MET	151.9±6.12*	106.5±7.09*	0.6486±0.045*
D-PIO+MET	201.2±8.07	124.4±11.14	0.9337±0.066

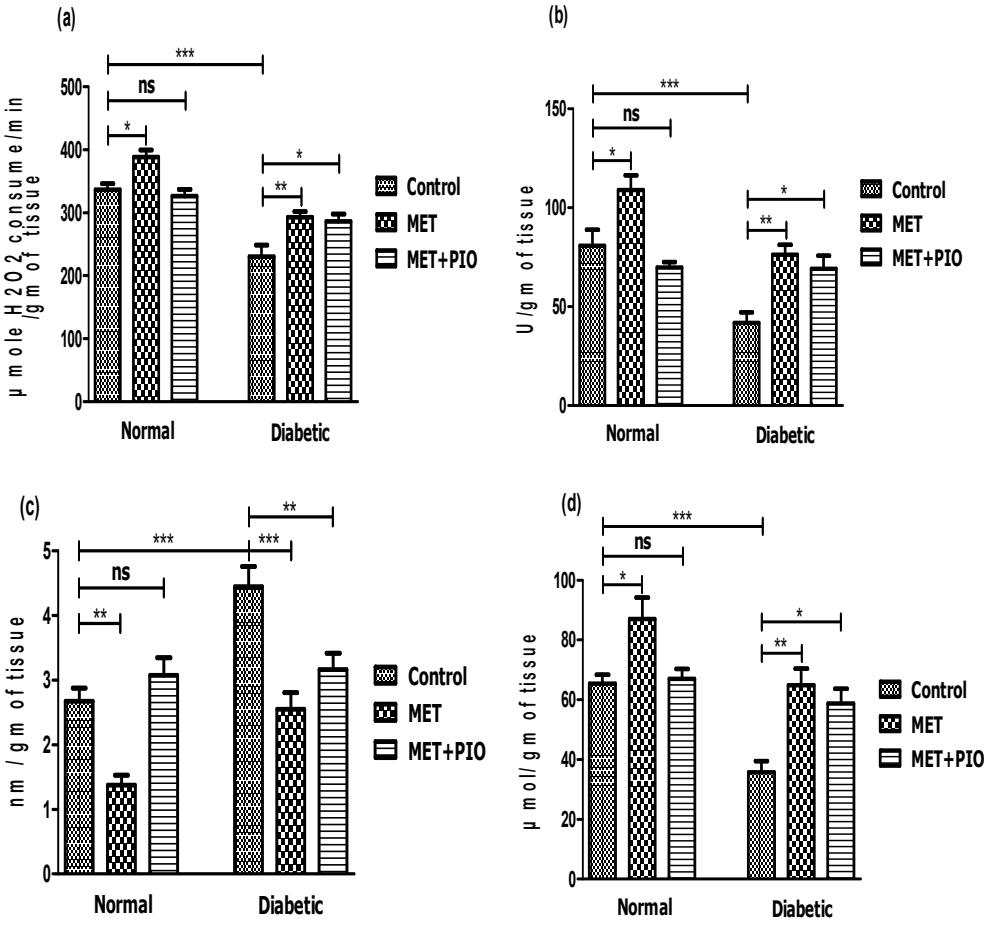
Values are expressed as mean ± SEM for six animals in the group. $^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$ considered statistically significant as compared to ND-CON group; $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.001$ considered statistically significant as compared to D-CON group

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 MET (50 mg/kg) again significantly (p<0.001) 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 MET (50 mg/kg) combination with PIO (10 mg/kg) for 4 weeks, showed significantly ($p<0.01$) decreased MDA and increased the levels of GSH ($p<0.05$), CAT ($p<0.05$) and SOD ($p<0.05$) changes in the tissue levels as compared to DB-CON group and NB-CON group.

Figure 2. Effect of Metformin and Pioglitazone on CAT (a), SOD (b), MDA (c) and GSH (d) level in non diabetic and diabetic rats.



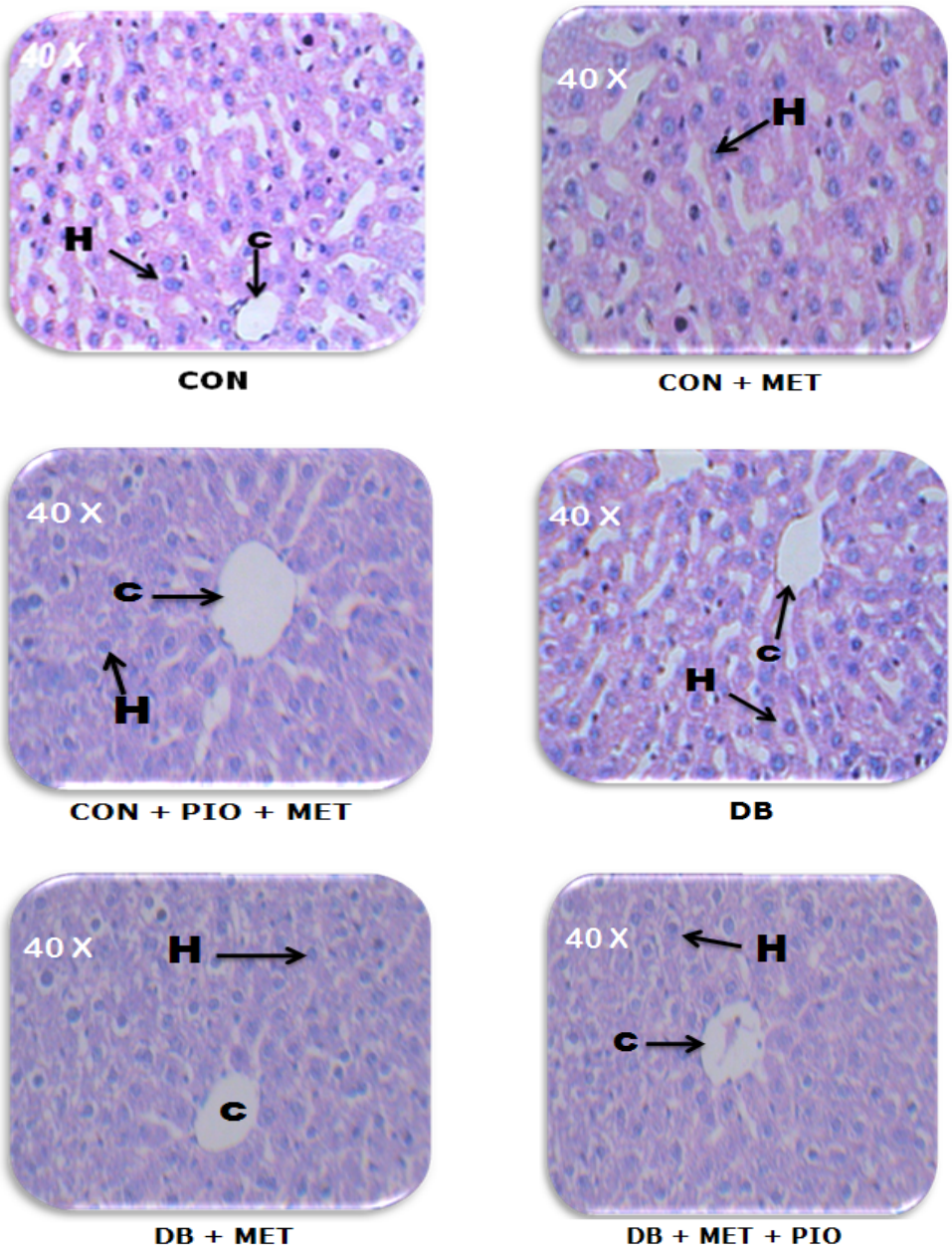
Values are expressed as mean ± SEM for six animals in the group. $^{*}P<0.05$, $^{**}P<0.001$; $^{***}P<0.001$ considered statistically significant as compared to Control group.

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. MET (50 mg/kg) combination with PIO (10 mg/kg) administration caused significant effects in the livers of both ND and DB rats. In the liver of ND-CON group, rats at 4 weeks after MET (50 mg/kg) alone and MET (50 mg/kg) with 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 MET (50 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 MET (50 mg/kg) with PIO (10 mg/kg) administration.

Figure 3. Effect of Metformin and Pioglitazone on Liver tissues in non diabetic and 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 MET (50 mg/kg) alone and its combination with PIO (10 mg/kg) 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 [16].

In STZ-NIC induced diabetes, the characteristic loss of body weight caused by an increase in muscle wasting [17]. In the present study treatment with MET with 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. Treatment with MET alone and along with PIO showed a significant decrease in the level of glucose and HbA1c as compared with DB-CON rats.

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 [18]. It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c [19].The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes [20].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 [21-23]. It has been reported that an increased level of transaminases under insulin deficiency [24] were responsible for the increased gluconeogenesis and ketogenesis during diabetes.

The increased levels of serum AST, ALT ALP have already been reported to be associated with liver dysfunction in diabetes [25]. Decreased in the activities of AST, ALT, ALP and γ- GTP in MET along with PIO treated rats indicate the protective role of the MET in combination with 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 [26].The effects of thiazolidinediones on oxidative stress are difficult to predict [27]. Previous studies have proved that, thiazolidinedione exposure increase oxidative stress [28]. SOD and CAT are considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species [29]. SOD is an important defense enzyme, which catalyzes the dismutation of superoxide radicals [30] and CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals [31]. 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 [32].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 [33-34]. In our study, the activities of SOD, CAT, GSH was significantly increased and the level of lipid peroxidation was significantly decreased with MET alone and in combination with PIO.

This study concluded that MET (50 mg/kg) alone and combination with PIO (10 mg/kg) may show some protection in STZ-NIC induced diabetic rats whereas with doses and chronic treatment it showed further liver protection but MET alone may be better than MET and PIO with combination 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. Dipesh P, Hiren NP, Kaushal P, Venkatraghavan S, Rajesh V, Leelathi DA, Sureshwar P. Continuing Pharmacy Education Series: Diabetes. The Indian journal of hospital pharmacy 2009; 46: 7-19.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. Verma S, Bhanot S, McNeill JH. Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J Pharmacol Exp Ther. 1994; 271:1334 –1337.
9. Jayesh B. Majithiya, Arvind N. Paramar, R. Balaraman. Pioglitazone, a PPARγ- agonist, restores endothelial function in aorta of streptozotocin-induced diabetic rats; Cardiovascular Research 2005:66:150– 161
10. 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.
11. 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.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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. Pioglitazone attenuates diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. Nephrology Dialysis Transplantation 2008 23(9):2750-2760.
17. 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.

18. Paulsen, E.P. Heamoglobin A1C in childhood of diabetes. Metabolism 1973; 22: 269- 271.
19. 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.
20. Gabbay, K.H. Glycosylated hemoglobin and diabetic control. New England Journal Medicine 1976; 95: 443-454.
21. 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.
22. 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.
23. 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.
24. Fleig. P., Marliss, E. Ohman, J. and Cahill Jr, J.F. Plasma amino acid levels in diabetic keto acidosis. Diabetes 1970; 19: 727- 729.
25. 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.
26. 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 α-Lipoic Acid. Diabetes 2001; 50:404-410.
27. 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
28. 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.
29. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press, 1985; p. 1-27.
30. 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.
31. Chance B, Greenstein DS, Roughton RJW. The mechanism of catalase action – steady state analysis. Arch Biochem Biophys 1952; 37:301-339.
32. 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.
33. Meister A. New aspects of glutathione biochemistry and transport selective alterations of Glutathione metabolism. Nutr Rev 1984; 42:397- 410.
34. Nicotera P, Orrenius S. Role of thiols in protection against biological reactive intermediates. Adv Exp Med Biol 1986; 97:41-49.