EFFECT OF PIOGLITAZONE ON LIVER FUNCTIONS IN NORMAL AND STREPTOZOTOCIN NICOTINAMIDE INDUCED DIABETIC RATS

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Summary

The present study was designed to evaluate the effects of Pioglitazone (PIO) on liver function in normal and streptozotocin nicotinamide induced diabetic in rats. Pioglitazone (1, 5, and 10mg/kg, p.o) was administered for 28 days in rats injected with single dose of streptozotocin (65 mg/kg, i.p, STZ) and nicotinamide (110 mg/kg, i.p, NIC). Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamic transpeptidase (γGTP), creatinine phosphokinase (CPK) and lipid peroxidation (LPO) whereas the levels of lactate dehydrogenase (LDH) and total bilirubin (TB) were found to be non significant. The activities of endogenous antioxidants such as reduced glutathione (GSH), catalase (CAT) and super oxide dismutase (SOD) were significantly decreased in STZ–NIC induced diabetic rats as compared to control rats. Treatment with PIO showed alteration in all the serum markers and biomarkers of oxidative stress compared to STZ–NIC treated animals. Among all the three doses of PIO,1mg/kg was found to safe, 5mg/kg and PIO 10mg/kg was found to elevate the condition i.e it further increases the liver toxicity produced by STZ–NIC treatment in rats. Histopathological changes are also in correlation with biochemical changes. This study indicates that PIO cannot be useful in STZ–NIC hepatic complications associated with diabetics in rats.

Keywords: Pioglitazone, Antioxidant, Hepatotoxicity, 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).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 (2, 3). PIO hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. PIO showed dose dependant beneficial effects in many of the pathological conditions including reduction in blood glucose, lowering blood pressure and restoring endothelial functions in animals (4). Troglitazone which is withdrawn from the U.S. market in 2000 because of his high incidence of hepatotoxicity and drug-induced liver failure (5).

Literature survey showed that, there was no report regarding the effect of PIO on liver function in diabetic rats. In light of above survey, the present study was designed to evaluate the effect of PIO on liver functions and biomarkers of oxidative stress in STZ-NIC induced diabetic rats.

Materials and method

Drugs and Chemicals

Pioglitazone hydrochloride was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, India. STZ and NIC were obtained 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 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*.

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 (6). After 7 days following STZ and NIC administration, blood was collected from tail vein and serum samples were analyzed. 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 animals treated with PIO (1 mg/kg/day, p.o, ND-PIO1).
Group 3: nondiabetic group treated with PIO (5 mg/kg/day, p.o, ND-PIO5).
Group 4: nondiabetic group treated with PIO (10 mg/kg/day, p.o, ND-PIO10).
Group 5: diabetic control, single injection of STZ (65 mg/kg, i.p) and NIC (110 mg/kg, i.p, D-CON).
Group 6: STZ-NIC diabetic rats treated with PIO (1 mg/kg/day, D-PIO1).
Group 7: STZ-NIC diabetic rats treated with PIO (5 mg/kg/day, D-PIO5).
Group 8: STZ-NIC diabetic rats treated with PIO (10 mg/kg/day, D-PIO10).

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 (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} week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose, HbA1c, AST, ALT and TB were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). Activities of ALP, γGTP, LDH and CPK were estimated using standard diagnostic kits (Crest Biosystems, India, Erba Diagnostic Germany Limited).

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 (7) Hugo Aebi as given by Hugo (8) Moron et al (9) and Mishra and Fridovich (10).

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.

As shown in table1, treatment with PIO (10 mg/kg, p.o) showed a significant (P<0.001) increase in body weight as compared to control non-diabetic (ND) rats and DB-CON rats. The levels of glucose and HbA1c was significant (P<0.001) decreased after treatment with PIO (5, 10 mg/kg, p.o) as compared to DB-CON rats.

Effect of PIO on serum marker enzymes

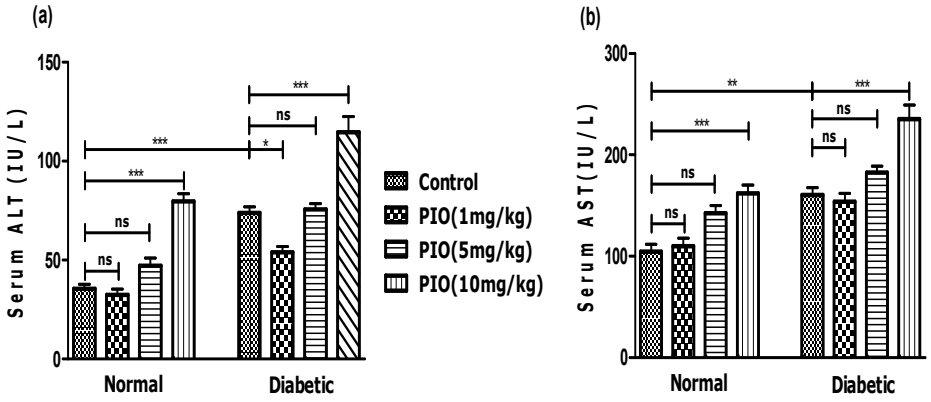
Figure 1 showed a significant (P<0.001) increase in serum AST and ALT levels in STZ-NIC treated rats (DB-CON) as compared to ND-CON animals. Treatment with PIO (10 mg/kg) for 4 weeks, showed further increase in serum AST and ALT level (P<0.001) as compared to DB-CON group alone. Whereas treatment with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

Table 1. Effect of Pioglitazone (1, 5 and 10 mg/kg/day, p.o) on changes in Body weight, serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.

Group	Body weight (gm)	Glucose (mg/dl)	Glycosylated heamoglobin (%, HbA1c)
ND-CON	248.33±5.95	101.0±15.13	5.45±0.37
ND-PIO1	256.00±6.31	74.59±14.03	5.16±0.36
ND-PIO5	270.17±7.67	70.23±18.23	4.35±0.30
ND-PIO10	300.00±12.87 $^{\$\$\$}$	60.75±16.93	4.023±0.25
D-CON	224.83±8.52	406.8±15.93 $^{\$\$\$}$	11.18±0.52 $^{\$\$\$}$
D-PIO1	247.83±7.77	305.9±18.33	9.61±0.40
D-PIO5	260.17±7.10	151.2±14.16 ***	6.67±0.26 ***
D-PIO10	265.50±8.11 ***	117.8±15.11 ***	5.77±0.2576 ***

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.

Figure1. Effect of pioglitazone (1, 5 and 10 mg/kg/day, p.o) on changes in serum ALT (a) and AST (b) level in normal and STZ-NIC induced diabetic rats.



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

Administration of STZ-NIC alone significantly increases (P<0.001) ALP, γGTP and CPK levels as compared to control rats but there was no significant changes in the levels of LDH and TB. As shown in table 2, treatment with (10 mg/kg, p.o) showed a significant (P<0.001) increase in ALP, γGTP, CPK and TB as compared to control ND rats and DB control rats only the exception was level of LDH which is increased in DB control rats. Administration of PIO (5mg/kg) significantly (P<0.05) increased ALP, γGTP and CPK levels compared to DB control. Treatment with PIO (5 mg/kg) showed a no significant changes in LDH and CPK levels as compared to DB control rats. Treatment with PIO (1mg/kg) showed non- significant changes in ALP, γGTP, LDH, CPK and TB as compared to DB control rats.

Table 2. Effect of Pioglitazone (1, 5 and 10 mg/kg/day, p.o) on changes in ALP, γGTP, LDH, CPK and TB level in normal and STZ-NIC induced diabetic rats.

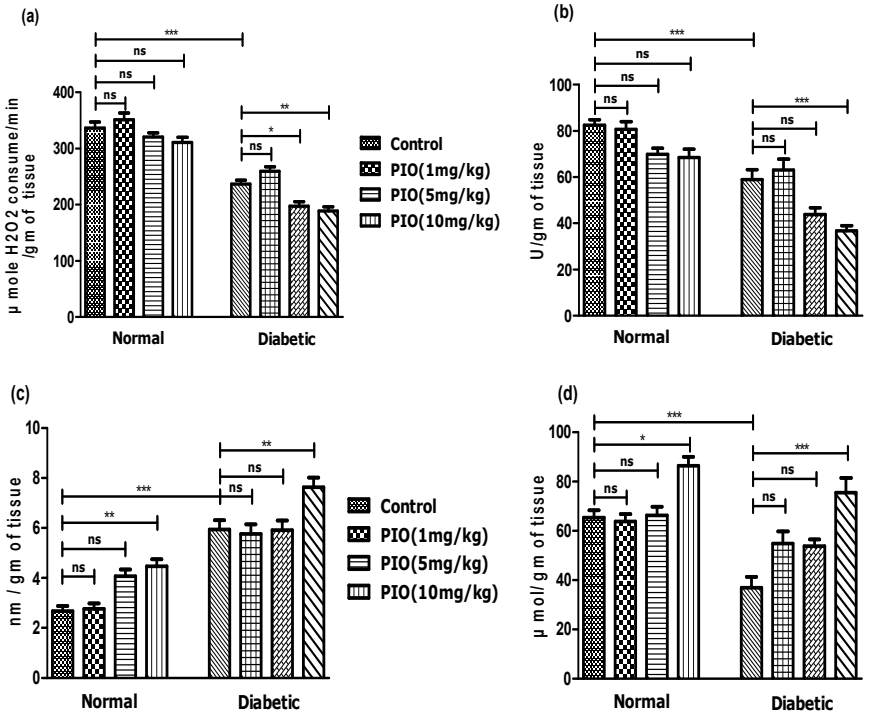
Group	ALP (IU/L)	γGTP (IU/L)	LDH (IU/L)	CPK (IU/L)	TB (IU/L)
ND-CON	138.2±7.43	75.24±4.45	340.4±9.36	91.50±5.04	0.719±0.041
ND-PIO1	171.0±7.17	91.62±3.10	325.6±9.15	100.2 ± 4.92	0.702±0.042
ND-PIO5	169.5±7.73	94.12±4.38	340.6±11.68	116.8 ± 7.97	0.816±0.056
ND-PIO10	257.1±4.33$^{\$\$\$}$	143.2±4.07$^{\$\$\$}$	379.7±7.55	167.0±7.77$^{\$\$\$}$	1.104±0.022$^{\$\$\$}$
D-CON	180.8±6.12$^{\$\$\$}$	92.36± 3.73$^{\$\$\$}$	369.5±10.90	111.8±8.22$^{\$\$\$}$	0.891±0.056
D-PIO1	203.0±8.18	112.2±5.75	359.4±8.82	125.9±5.83	0.701±0.052
D-PIO5	217.2±8.481*	117.3± 5.87*	405.8±9.41	141.2 ± 6.23	1.190± 0.110*
D-PIO10	292.0±7.54***	141.4±5.09***	459.9±10.01***	196.0±6.23***	1.901 ±0.059***

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 PIO (10mg/kg) again significantly ($p<0.01$) increased MDA and decreased the levels of GSH ($p<0.001$), CAT ($p<0.01$) and SOD ($p<0.001$) (Fig. 2).

Figure 2. Effect of pioglitazone (1, 5 and 10 mg/kg/day, p.o) on changes in CAT (a), SOD (b), MDA (c) and GSH (d) level in normal and STZ-NIC induced diabetic rats.



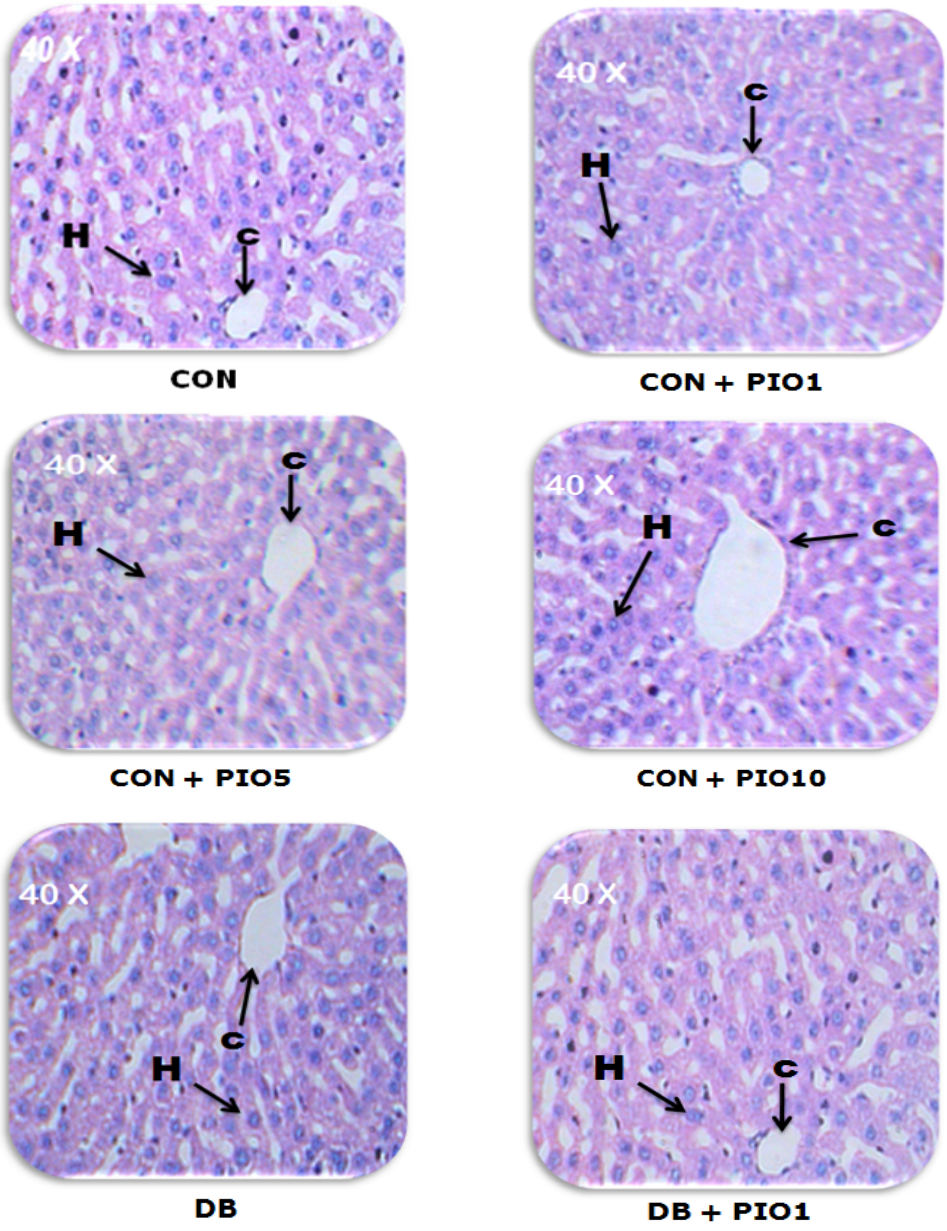
(a) Catalase (CAT) b) Superoxide dismutase (SOD) c) Lipid Peroxidase or malondialdehyde (MDA) d) reduced glutathione (GSH) levels in rats subjected to after 4 weeks, Values are expressed as mean ± SEM for six animals in the group. $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.001$ considered statistically significant as compared to Control group.

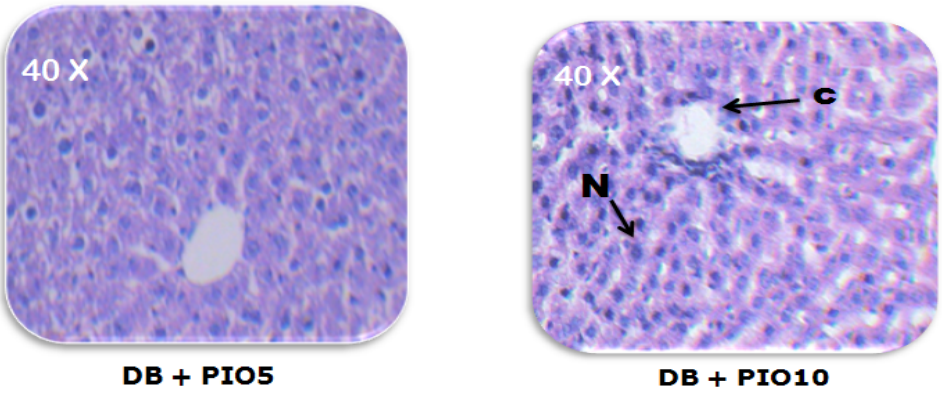
Effect of PIO on Histopathological changes

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. PIO 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 PIO (1 & 5mg/kg) administration showed swollen hepatocytes with inflammatory cell infiltration in centrilobular area were minimally evident.

Vacuolar degeneration and centrilobular necrosis of hepatocytes and mild inflammation were observed at 4 weeks after PIO (10 mg/kg) administration. In contrast, in the liver of DB rats, mild to moderate coagulative necrosis was evident at 4 weeks after PIO (5 mg/kg) administration. These effects were progressive by 4 weeks after PIO (10 mg/kg) administration, manifesting severe to massive coagulative necrosis with extensive bridging of necrotic zone affecting all lobules. The liver of DB rats at 4 weeks after PIO (1mg/kg) administration showed swollen hepatocytes with inflammatory cell infiltration in centrilobular area was minimally evident.

Figure 3. Effect of Pioglitazone (1, 5 and 10 mg/kg/day, p.o) on changes in Liver tissues in normal and STZ-NIC induced diabetic rats.





C = Central vein, **N** = Hepatocytes Necrosis, **H** = Normal Hepatocytes

Discussion

The present study was under taken with the objective of exploring the hepatic function of PIO in STZ-NIC induced diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Peroxisome proliferator-activated receptors are nuclear transcription factors that play a role in insulin sensitivity (11).

In STZ-NIC induced diabetes, the characteristic loss of body weight caused by an increase in muscle wasting (12). In the present study treatment with PIO (10 mg/kg) 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 PIO (5 and 10 mg/kg) 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 (13). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (14).The level of HbA1c is always monitered as a reliable index of glycemic control in diabetes (15).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, and ALP, γ- GTP and CPK (16-18). It has been reported that the increased levels of transaminases under insulin deficiency (19) 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 to liver dysfunction and leakage of these enzymes to the liver cytosol in to the blood stream in diabetes (20).

Enhanced in the activity of AST, ALT, and ALP, γ- GTP and CPK in PIO (10 mg/kg) treated diabetic rats indicate the alleviating role of the 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 (21). The effects of thiazolidinediones on oxidative stress are difficult to predict (22). Previous studies have proved that, thiazolidinedione exposure increase oxidative stress (23). SOD and CAT are considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species (24). SOD is an important defense enzyme, which catalyzes the dismutation of superoxide radicals (25) and CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (26). 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 (27). 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 (28, 29). In our study, the activity of endogenous antioxidants was not significantly changed with PIO (1 & 5 mg/kg). Treatment with PIO 10mg/kg further decreases the levels of endogenous antioxidants and increases the level of lipid peroxidation.

This study concluded that PIO at 1 and 5mg/kg may show some protection in STZ-NIC induced diabetic rats whereas with high doses and chronic treatment it showed further liver damage.

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