Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2010, 2 (1) 95-103
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Liver Injury Induced by Renal Ischemia Reperfusion in Diabetes Type-II

Shivanand Pandey*

Smt. R.B.P.M.P.C., Atkot, Rajkot, Gujarat, India

Abstract

This study was designed to investigate the effect of diabetes on liver injury (distant organ) induced by renal Ischemia Reperfusion injury (IR) in rat. Diabetes Mellitus type-II (DM-II) in rats were induced by administration of nicotinamide (230mg/kg, i.p.), 15 min prior, to the single dose of streptozotocin (STZ) (65mg/kg, i.v.). In vivo renal IR was performed in both type-2 diabetic and normal rats. Each protocol comprised ischemia for 30 min followed by reperfusion 24 hrs. The lipid peroxidation, xanthine oxidase activity, myeloperoxidase activity and nitric oxide level in liver tissue were significantly increased after IR in diabetic rats compared to non-diabetic rats. Antioxidant enzymes like glutathione superoxide dismutase, catalase and glutathione peroxidase were significantly reduced after IR in diabetic rats compared to normal rats. Diabetes type-II had exaggerated remote organ injury (liver) induced by renal IR injury in diabetes.

Keywords: Diabetes type-II; Ischemia reperfusion; Kidney; Liver injury; Oxidative stress

---

Introduction

Diabetes mellitus (DM) increases renal sensitivity to ischemia reperfusion (IR) injury [1]. DM causes organ dysfunctioning and increases the sensitivity of organs to damages. Diabetic patients may need renal transplantation in their later life due to diabetic nephropathy. The IR injury is one of the dangerous complications of this procedure. The short period of ischemia (30 min) in diabetes has been demonstrated to reversible renal failure, leading to progressive injury with end stage renal disease [2]. The various investigators have reported that renal IR causes distant organ injury such as liver injury [3, 4, 5].

Reactive oxygen species (ROS) and nitric oxide (NO) play an important role in mediating cell damage during IR injury [6, 7]. Inflammation contributes substantially to the pathogenesis of IR

with a central role for particular cells, adhesion molecules, and cytokines [8]. Neutrophils are the inflammatory cells, which produces abundantly ROS during IR injury. Myeloperoxidase (MPO) is found in neutrophils and catalyzes the formation of hypochlorous acid (HOCl), a toxic agent to cellular components and initiates oxidative injury [9]. Renal IR causes tissue injury by oxygen radicals and oxidative stress caused by an imbalance between production of ROS and the antioxidant capacity [10].

Liver injury is one of the distant organ damage induced by kidney IR. Acute renal failure associated with liver disease is a commonly encountered clinical problem of varied etiology. It is believed that IR injury induces inflammatory response, that elicits tissue damage in a number of organs in which reactive oxygen and nitrogen species play a key role in the pathophysiology of renal IR injury [10, 11]. It demonstrated that renal IR injury might cause liver oxidative stress and increase lipid peroxidation in liver tissue [12]. The liver tissue of rat decreases antioxidant enzyme activities after renal IR is well reported [5].

Diabetic patients may need renal transplantation in their later life due to diabetic nephropathy and it can cause multiple organ damages. So, Present work designed to understand the effect of DM-II on liver injury induced by renal IR.

Materials and Methods

Induction of Diabetes Type-II

Healthy adult wistar rats (either sex) weighing 200-250g were used. The experiment and protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) of Smt. R.B.P.M.C. Atkot and with permission from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Diabetes Mellitus type-II (DM-II) in rats were induced by administration of nicotinamide (230mg/kg, i.p.), 15 min prior, to the single dose of streptozotocin (STZ) (65mg/kg, i.v.) [13]. Control animals were received an equal volume of saline. The STZ solution was contained STZ in saline with a sodium citrate buffer, pH 4.0. Food, water consumption, weight gain and the blood glucose levels were recorded to monitor the degree of diabetes. Four weeks were elapsed in between the induction of diabetes and ischemic injury.

Induction of Renal IR Injury in DM-II

Diabetic rats were anesthetized with ketamine (60 mg/kg i.p.) and diazepam (5 mg/kg i.p). Body temperature was maintained throughout surgery at 37±0.5°C. The skin on back was shaved and disinfected with povidone iodine solution. All rats were undergoing surgical exposure of the left and right renal pedicles via midline incision. To induce renal ischemia, both renal pedicles were occluded for 30 min with vascular clamps. After 30 min of occlusion, the clamps were removed, and kidneys observed to undergo reperfusion for 24 hrs. Rats were randomly divided into three different groups (n=6) (Figure 1). Group 1: Normal control, Group 2: Renal IR injury, Group 3: Diabetes + Renal IR injury. At the end of each in vivo study, the rats were sacrificed and liver were quickly removed and placed into liquid nitrogen and then stored at -70^{o}C until assayed for oxidant and antioxidant parameters.

Estimation of Liver Function

Estimation of liver function was carried out by measuring marker enzymes of liver function like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) by using kit (Span Diagnostic Ltd, India)

Estimation of Lipid Per-oxidation and Antioxidant Enzymes

The liver was removed and kept in cold conditions (precooled in inverted petridish on ice). It was cross chopped with surgical scalpel into fine slices in chilled 0.25 M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10%w/v) with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for assays of lipid peroxidation (MDA content) and endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH)) and Glutathione peroxidase (GSHPx). MDA formation was estimated by the method of Slater and Sawyer [14]. Reduced glutathione was determined by the method of Moron et al (Moron et al., 1989) [15]. Superoxide dismutase was determined by the method of Mishra and Fridovich (Mishra and Fridovich, 1972) [16]. Catalase was estimated by the method of Levine RL et al. [17]. Glutathione peroxidase was determined by the method of Paglia and Valentine [18].

Determination of Xanthine Oxidase Activity

Tissue xanthine oxidase (XO) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm (Prajda and Weber, 1975) [19]. The phosphate buffer (pH 7.5) and xanthine was mixed with supernatant sample and then incubated for 30 min at 37^{o}C. The reaction was stopped at 0 and 30 min by addition of 100% trichloroacetic acid. Then, the mixture was centrifuged at 5000 g for 30 min. The activity was measured at 293 nm. One unit of activity was defined as 1 mmol of uric acid formed per minute at 37^{o}C, pH 7.5.

Determination of Nitric Oxide Level

The nitrite (NO) was estimated by the method of described previously [20]. To 0.5 ml of tissue homogenate, 0.1 ml of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for estimating nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained. Standard curve was prepared by sodium nitrite solutions with concentrations in range 1–100 µM by diluting the nitrite standard solution.

Determination of Myeloperoxidase Activity

MPO activity was measured in tissues in a procedure similar to that documented by Wei and Frenkel [21]. Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41,000 g (10 min); pellets were suspended in 50 mM phosphate buffer containing 0.5 % hexadecyltrimethylammonium bromide (HETAB). Aliquots (0.3 mL) were

added to 2.3 mL of reaction mixture containing 50 mM Phosphate buffer, o-dianisidine, and 20mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/gm of tissue.

Statistical Analysis

All the values are expressed as mean ± SEM. Statistical significance between more than two groups were tested using one-way ANOVA followed by the Bonferroni multiple comparisons test using computer based fitting program (Prism, Graphpad 5.). Differences were considered to be statistically significant when $P < 0.05$.

Results

Effect of DM-II on Liver Function

Diabetic rats that underwent renal IR in exhibited significant increase in the serum concentrations of ALT, AST and ALP as compared to non-diabetic rats ($P<0.001$), suggesting a significant degree of liver dysfunction caused by renal I/R in diabetes (Figure 2A-C).

Figure 1

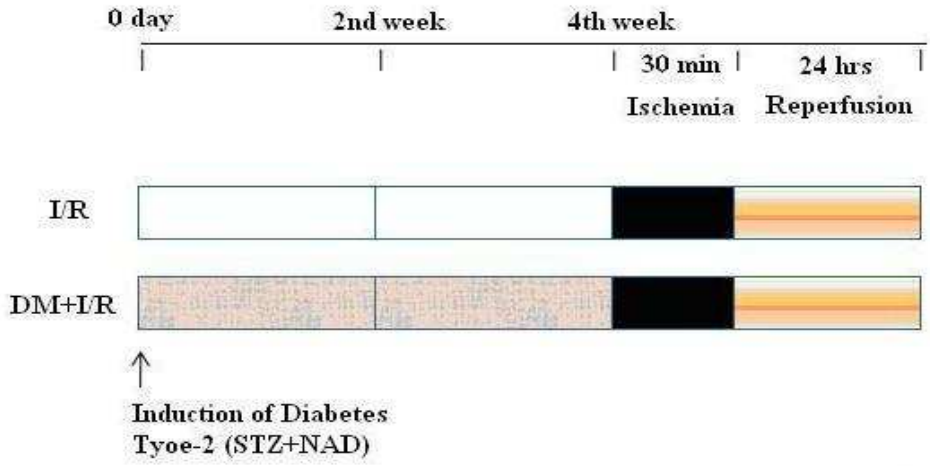


Figure2

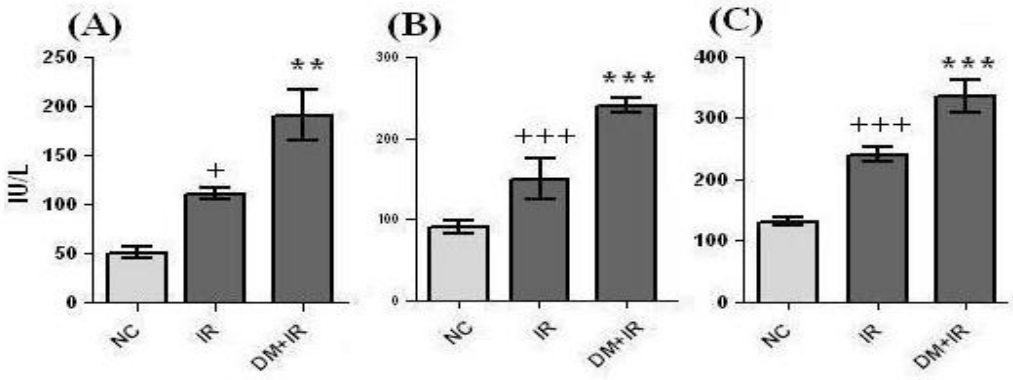
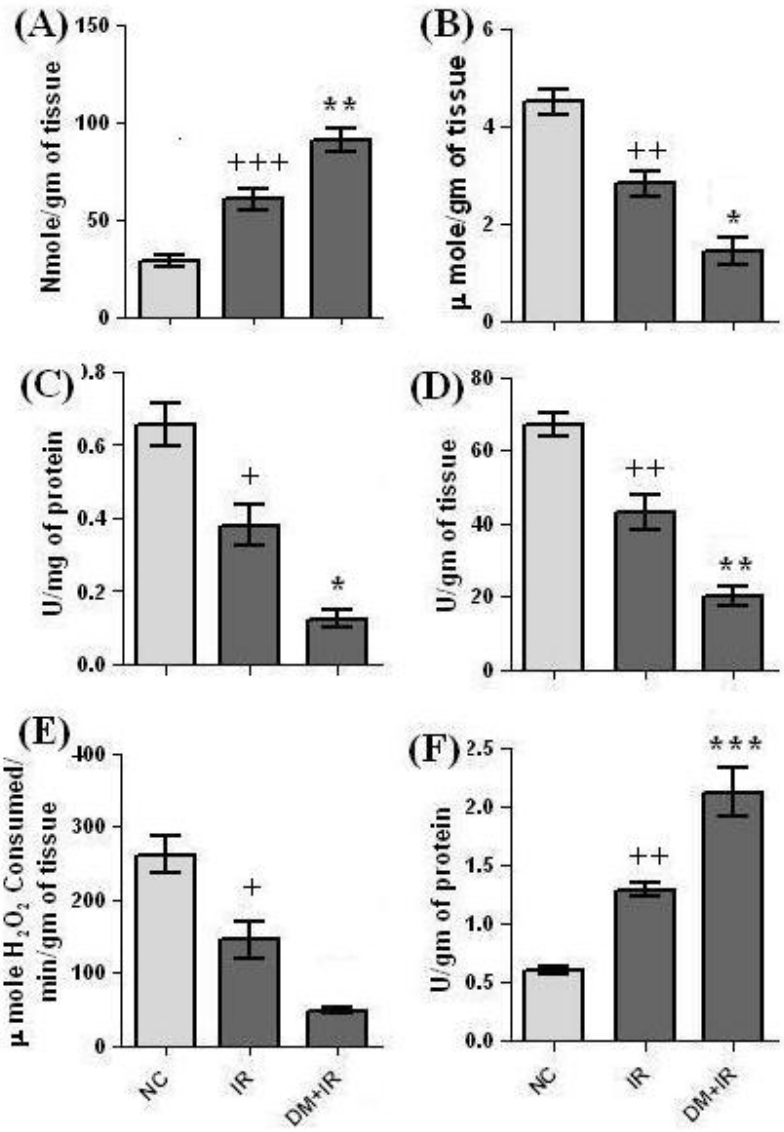


Figure 3



Effect of DM-II on Lipid Peroxidation and Antioxidant Enzymes

The MDA level in liver tissue, was significantly increased in diabetic IR group compared to non-diabetic IR ($P<0.01$) (Figure 3A) and significant decrease was found in the level of GSH ($P<0.05$) as well as in the activity of SOD ($P<0.01$) (Figure 3B, 3D) in the diabetic IR group as compared to non-diabetic IR group. Diabetic group demonstrated a significant decrease in GSHPx activity after IR compare to control group, and significant difference was observed in between non-diabetic IR rats and diabetic IR rats ($P<0.05$) (Figure 3C). The CAT activity did not alter in diabetic IR group compared to diabetic group (Figure 3E).

Effect of DM-II on Xanthine Oxidase Activity

The XO enzyme activity, one of the sources of ROS production, was significantly (P<0.001) increased in diabetic IR group compare to normal control. In the diabetic IR group XO activity significantly (P<0.001) increased compare to non-diabetic IR rats (Figure 3F).

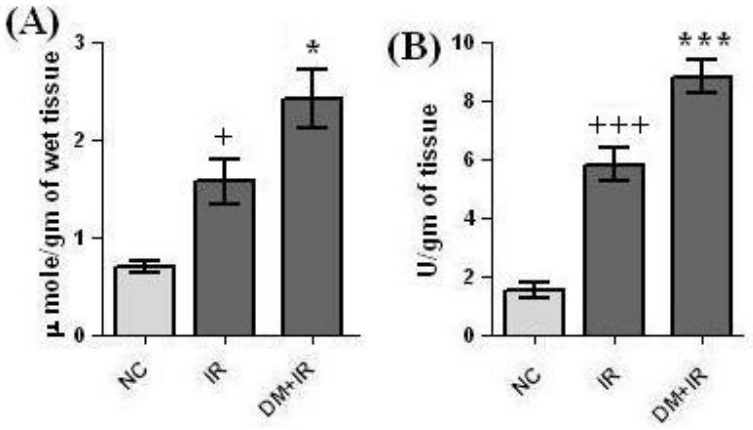
Effect of DM-II on Nitric Oxide level

The level of NO was significantly increased in non-diabetic IR with normal control (P<0.05). Diabetic IR group had significant (P<0.05) high NO level as compare to non-diabetic IR group (Figure 4A).

Effect of DM-II on Myeloperoxidase Activity

Myeloperoxidase activity, which is accepted to be an indicator of neutrophil infiltration, was significantly higher in the liver tissue of the diabetic IR group than in the liver tissue of the non-diabetic IR group (P < 0.001) (Figure 4B).

Figure4



Discussion

In the present study, ALT, AST and ALP activities did not increase after renal IR as much as a liver failure. However, their statistically significant rise was found in the liver function enzymes after renal IR in diabetic rats than non-diabetic rats, which indicated severe diminished liver function, in diabetes than normal. That might be attributed to diabetes had potentate liver injury induced by renal IR. An important question in this work is how DM-II could cause the increased sensitivity to liver injury induced by renal IR, which observed in DM-II animals. Several possible explanations exist. The increased sensitivity to damage by IR could be due to hyperglycemia per se. Shortage of insulin could also be involved in the increased sensitivity to liver injury. Secondary effects of hyperglycemia such as formation of advanced glycosylated end products, increased oxidative stress, hemodynamic alterations, and formation of NO could also be involved.

We found significant higher MDA level in the liver tissue of both non-diabetic and diabetic rats after induction of renal IR injury, which is major index of lipid peroxidation and oxidative stress. This might be due to ROS production via inflammatory response as inflammatory reactions are activated during the process of IR injury, resulting in the formation of inflammatory cytokines, like tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and arachidonic acid metabolite. Cyclooxygenase (COX)-2 is induced in response to pro-inflammatory cytokines and it catalyzes the metabolism of arachidonic acid. It reported that from 3 to 5 h after IR injury COX-2 expression was most intense and from 12 to 24 h after IR injury maximal tissue damage was observed. Thus, we decided to analyze tissue injury after 30 min of ischemia and 24 h of reperfusion [22]. Demonstration of lipid peroxidation helps to explain better exact mechanism of renal IR on liver tissue and it was found significantly higher in this study, which indicated generation of oxidative stress.

The cardiac MPO activity increased after renal IR, consistently with leukocyte infiltration and activation. The active neutrophils show high MPO activity in the tissue as an inflammatory answer. Present work shown that liver MPO activity was higher in non-diabetic IR group and further increased in diabetic IR group similar to those cardiac results. The finding that liver MPO activity was increased after induction of IR is very important because it clearly shows high leukocyte function in the liver tissue. The neutrophils play a major role in oxidant injury via the mechanisms such as the action of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or MPO system. Hypochlorous acid is produced largely from stimulated neutrophils by MPO activity. Hypochlorous acid causes oxidation of other molecules such as proteins, amino acids, carbohydrates, nucleic acids and lipids, expanding liver tissue damage.

Another radical producing mechanism might be NO producing system and the reaction of NO with $O2^{\bullet-}$ results in peroxynitrite formation, a potent and aggressive cellular oxidant and causes the formation of 3-nitro-L-tyrosine [23]. Nitrite/nitrate levels, as the end products of nitric oxide conversion, were increased in blood plasma and aortic tissue in diabetic animal's comparison with non-diabetic animals, which was confirmed by elevated NO level in our study. Streptozotocin-induced diabetes caused increased in activity and expression of liver iNOS. NO levels found to be significantly elevated in diabetic liver tissue at a very early stage in the investigation of Stadler et al. Present results have demonstrated the involvement of iNOS in the inflammatory process and might have a role in distant organ injury induced by renal IR via activated iNOS producing cells. We found high NO level in diabetic IR rats compared to non-diabetic IR rats and that was same as reported previously [24]. Liver tissue from the diabetic group did not show any evidence of the occurrence of ROS (Evelson et al., 2005) [24] those data are in good agreement with our finding. Increased NO production in DM did not alter cellular function in liver tissue. Also, DM did not affect the serum liver enzymes ALT and AST in comparison with control group.

The results of present work indicated that DM-II caused increase in lipid peroxidation in liver tissue after renal IR. Antioxidant enzymes like GSH, GSHPx, CAT and SOD were decreased in liver tissue followed by renal IR in diabetic rats. Also DM had elevated MPO activity. Thus, DM exaggerated liver injury by neutrophil activation and ROS production as well as increase in XO activity. Some previous works support the importance of BGC in IR injury. In our study we found severe liver injury when IR performed in DM-II rats, in which blood glucose

concentration (BGC) was higher than in the normal rats. Hyperglycemia, the elevated BGC during I/R could be deleterious for the liver injury. An increased acute sensitivity to ischemia has been demonstrated when BGC was raised by dextrose infusion or intraperitoneal glucose injection in combination with renal I/R in both rats and dogs [25]. Numerous studies have investigated the influence of hyperglycemia and diabetes in cerebral ischemia. Diabetes is associated with a worse outcome after stroke in humans, and elevated blood glucose predisposes for a more severe cerebral injury even in non-DM patients (Pulsinelli WA et al., 1983) [26]. There are conflicting evidences regarding the influence of hyperglycemia and diabetes on the degree of injury in experimental cerebral ischemia. DM or hyperglycemia in non-DM animals caused increased cerebral injury in most studies, especially when models with reperfusion were used. Taken together these studies suggested a role for reperfusion in the harmful effect of hyperglycemia in cerebral ischemic injury, hyperglycemia might be a reason for sever liver injury in DM-II (Table 1).

Table: 1 Blood glucose concentration during the experiments

Groups	NC	I/R	DM+I/R
BGC (mmol/L)	4.8 ±0.94	4.2 ±0.84	32.58 ±2.51***

Values are mean ± SEM (n=6), analyzed by one way ANOVA followed by Bonfferoni's multiple comparison tests. *** p<0.001 Vs. IR.

Conclusion

In conclusion, diabetes has exaggerated liver damage induced by renal IR via elevation of oxidative stress and inflammatory process in STZ-NAD induced diabetic rats.

References

[1] Goor Y, Peer G, Iaina A, Blum M, Wolman Y, Chernihovsky T, Silverberg D, Cabili S (**1996**). *Diabetologia*. 39: 1036-1040.
[2] Melin J, Hellberg O, Akyurek LM, Kallskog O, Larsson E, Fellstrom BC (**1997**). *Kidney Int*. 52: 985-991.
[3] Serteser M, Koken T, Kahraman A, Yilmaz K, Akbulut G, Dilek ON (**2002**). *J. Sur. Res*. 107: 234-240.
[4] Emre MH, Erdogan H, Fadillioglu E (**2006)**. *Gen. Physiol. Biophys*. 25: 195-206.
[5] Sural S, Sharma RK, Gupta A, Sharma AP, Gulati S (**2000)**. *Ren. Fail*. 22: 623-634.
[6] Basireddy M, Isbell TS, Teng X, Patel RP, Agarwal A (2006). *Am. J. Physiol. Renal. Physiol*. 290: F779-F786.
[7] Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, Brodsky S, Goligorsky MS (**2001)**. *Am. J. Physiol. Renal Physiol*. 281: F948-F957.
[8] Ysebaert DK, De Greef KE, De Beuf A, Van Rompay AR, Vercauteren S, Persy VP, De Broe ME (**2004**). *Kidney Int.* 66: 491-496.
[9] Altunoluk B, Soylemez H, Oguz F, Turkmen E, Fadillioglu E (**2006**). *Ann. Clin. Lab. Sci*. 36: 326-332.
[10] Erdogan H, Fadillioglu E, Yagmurca M, Ucar M, Irmak MK (**2006)**. *Urol. Res*. 34: 41-46.

[11] Kelly KJ (**2003**). *J. Am. Soc. Nephrol*. 14: 1549-1558.
[12] Yildirim A, Gumus M, Dalga S, Sahin YN, Akcay F (**2003**). *Ann. Clin. Lab. Sci*. 33: 459-464.
[13] Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D (**1998)**. *Diabetes.* 47: 224-9.
[14] Slater TF, Sawyer BC (**1971**). *Biochem J.* 123: 805–814.
[15] Moran MS, Depierre JW, Mannervik B (**1979**). *Biochimica et Biophysica* ACTA. 582: 67.
[16] Mishra HP, Fridovich I (**1972**). *J Biol.Chem*. 247: 3170.
[17] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stantman ER (**1990**). Determination of carbonyl content in oxidatively modified proteins, In Packer, L. and Glazer, A.N. (Eds), Methods in Enzymology, Oxygen radicals in biological systems. Academic Press California. 186: 464-478.
[18] Paglia DE, Valentine WN (**1967**). *J Lab Clin Med*. 2: 158.
[19] Prajda N, Weber G (**1975)**. *FEBS Lett*. 59: 245-249.
[20] Guevara I, Iwanejko J, Dembinska-Kiec A (**1998**). *Clin Chim Acta.* 274: 177–88.
[21] Wei H, Frenkel K (**1993)**. *Carcinogenesis.* 14: 1195-1201.
[22] Matsuyama M, Yoshimura R, Hase T, Kawahito Y, Sano H, Nakatani T (**2005)**. *Transplant. Proc*. 37: 370-372.
[23] Yagmurca M, Erdogan H, Iraz M, Songur A, Ucar M, Fadillioglu E (**2004**). *Clin. Chim. Acta.* 348: 27-34.
[24] Evelson P, Susemihl C, Villarreal I, Llesuy S, Rodriguez R, Peredo H, Lemberg A, Perazzo J, Filinger E (**2005***). Ann. Hepatol*. 4: 115-120.
[25] Moursi M, Rising CL, Zelenock GB, D'Alecy LG (**1987***) Arch-Surg*. 122:790-794.
[26] Pulsinelli WA, Levy DE, Sigsbee B, Scherer P, Plum F (**1983**). *Am J Med*. 74:540-544.