

Alpha-Lipoic Acid and N-Acetyl Cysteine Ameliorates Oxidative Stress and Hepatic Injury in Alloxan-Induced Diabetic Rabbits

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Abstract Insulin dependent diabetes mellitus is a chronic metabolic disorder that continues to present a major worldwide health problem. It characterized by high level of glucose in blood due to deficiency of insulin. Generation of ROS is thought to mediate the cytotoxic action of alloxan on the pancreatic β -cell and play an important role in the complication of diabetes. The aim of the present work to evaluate the efficacy of antioxidant α -lipoic acid and N-acetylcysteine to ameliorate the oxidative stress in hepatic tissue that induced by alloxan treatment in rabbits. Thirty-two male white rabbits were used in the present study classified into four groups. Group worked as normal control group, 2nd worked as untreated diabetic rabbits, 3rd and 4th groups diabetic rabbits treated with α -lipoic acid and N-acetyl cysteine respectively. Diabetic rabbits showed weigh loss, increase in relative liver weight, plasma glucose level and ALT activity. Also, showed morphological changes in the liver with increased in LPO, NO, CP and DNA fragmentation % and alteration in the non-enzymatic antioxidant levels (Vit E and GSH) and enzymatic antioxidant activities (CAT, SOD, GPx, GR, GGT, and GST) compared to control. Treatment of diabetic rabbits with either α -lipoic acid or N-acetyl cysteine overcome the loss of body weight gain and normalized relative liver weight, plasma glucose and ALT activity. Moreover, treatment improves the oxidative stress parameters and morphological changes that induced by alloxan and hyperglycemia. Conclusion: oxidative stress in alloxan induced diabetic rabbits plays a crucial role in hepatic degenerative changes. Treatment of diabetic rabbits with α -lipoic acid or N-acetyl cysteine caused normoglycemia and liver cell reprogramming due to its antioxidant activities. Beneficial action seems to result mainly from direct scavenging of ROS and restoring GSH redox state.

Keywords Diabetes, Alloxan, A Lipoic Acid, N-Acetyl Cysteine, Oxidative Stress, Liver and Rabbits

1. Introduction

Alloxan the best known B-cell toxin, inside the cells, its central carbonyl is reduced by cellular thiols into dialuric acid and generate superoxide, hydrogen peroxide and hydroxyl radical[1]. This oxidative decomposition of dialuric acid is thought to account for alloxan toxicity in the B-cell, which has low levels of superoxide dismutase and glutathione[2]. Generation of ROS also, plays an important role in the etiology of diabetic complications. Under diabetic conditions, ROS are produced via glucose autooxidation[3]. Oxidative damage occurs as a consequence of imbalance between the formation and inactivation of oxygen free radicals. Inactivation and removal of ROS depend on relations with antioxidative defense mechanism. Oxidative damage leads to the destruction of membrane lipids and production

of lipoperoxides and their products[4].

α -lipoic acid is a disulfide compound that functions as a coenzyme in puruvate dehydrogenase and α -ketoglutarate dehydrogenase mitochondrial reactions, leading to production of cellular energy. α -lipoic acid reduce oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains by preventing membrane lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as Vit C and E and by increasing intracellular GSH[5&6]. Moreover, α lipoic acid improves the imbalance between increased oxidative stress and delpeted antioxidant defense even in patients with poor glycemic control and albuminuria[7].

N-acetyl cysteine is a small molecule containing a thiol group. In addition to its antioxidant properties, has the capacity to inhibit several inflammatory elements related to oxidative stress[8]. It prevents short-term hyperglycemia-induced insulin resistance *in vivo*[9], counteracting ethanol-induced oxidative stress[10] and has heptoptroective actions in chronic inflammatory liver disease which mediated partially through the modulation of nitric oxide produc-

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Published online at <http://journal.sapub.org/diabetes>

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tion[11].

In this study we aimed to determine the effects of α -lipoic acid and N-acetyl cysteine on the oxidative stress parameters in plasma and hepatic tissue and the hepatic morphological changes of diabetic rabbits induced by alloxan.

2. Materials and Methods

i- Animals and experiment design

Thirty two healthy male white rabbits of poskit strain with body weight average 800-1200 g were purchased from the farm of Faculty of Agriculture, Assiut University, Assiut, Egypt. They were housed in the animal house of the Zoology Department, Faculty of Science, Assiut University in wooden cages in groups of five rabbit per cage and were kept in a room temperature with normal light/dark cycle. Rabbits given commercial concentrated fodder: crude protein 17%, crude fat 2.63% and energy 2500 kcal (Al-wagh Alkbli company, Abnoub, Assiut, Egypt) and water *ad libitum* through the study period. All experimental protocols held on animals were done according to regulations set by the Institutional Animal Care and approved by Assiut University.

ii- Experimental design

After adaptation in the Animal House of Zoology Department for one week, the rabbits were randomly divided into two categories. The first contain 8 rabbits work as control group injected with a single dose of distilled water (1 ml/rabbit, i.p.). The second category treated animals contain 24 rabbits were injected with a single dose of alloxan (180 mg/kg body weight, i.p.)[12]. Then after three weeks (onset of type 1 diabetes) the rabbits were divided into three groups

- The first treated : was left as positive diabetic rabbits
- The second treated: was injected with α -lipoic acid (100 mg/kg body weight, i.p.) daily for 7 days.
- The third treated: was injected with N-acetyl cysteine (100 mg/kg body weight, i.p.) daily for 7 days.

- All animals were injected in similar volume injections

All experimental protocols held on animals were done according to regulations set by the Institutional Animal Care and approved by Assiut University.

ii- Samples collection

After the experimental period the animals were killed for collection of blood and liver was excised immediately, washed in ice cold saline. A portion of the liver was immediately utilized for histological and immunohistochemistry for iNOS. The remainder of liver was immersed in nitrogen and stored in -20°C and used for further studies. A 10% homogenate of liver was prepared in phosphate buffer (0.1M, pH 7.2).

iii- Biochemical measurements

Plasma glucose level was determined by enzymatic colorimetric method by using commercial kits (Diamond Diagnostics Company, Egypt) according to Trinder[13]. ALT

activity in plasma was assayed by commercial kit (Spectrum Diagnostics, Egypt) according to Young[14].

A total protein was measured by the method of Lowry *et al.*[15]. Nitric oxide was measured as nitrite concentration colorimetrically by the method of Ding *et al.*[16]. Lipid peroxides as thiobarbituric acid reactive substances was determined according to the method of Ohkawa *et al.*[17]. Carbonyl protein was determined as described by Stadtman and Levine[18]. Measurement of DNA fragmentation was based on the hydrolysis of DNA leading to release of free deoxyribose that was colorimetrically measured at 575nm after reaction with the dipenylamine reagent according to Kurita-Ochiai *et al.*[19]. Superoxide dismutase activity was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to Misra and Fridovich[20]. Catalase activity was estimated by procedure of Luck[21], basing on its ability to decompose H_2O_2 . Glutathione S-transferase activity was assayed using 1-chloro-2,4-dinitrobenzene as substrate according to the method of Habig *et al.*[22]. γ -glutamyl transpeptidase activity was determined by the method of Meister *et al.*[23] using γ -glutamyl p-nitroanilide as substrate. The activity of glutathione peroxidase was assayed by the method of Mohandas *et al.*[24]. Glutathione reductase activity was determined by the method of Carlberg and Mannervik[25]. Glutathione was determined using the method of Beutler *et al.*[26]. Vit E was measured using Emmerie-Engel reaction based on the reduction of ferric to ferrous ions forming a red complex with α,α -dipyridyl according to the method of Roe[27].

iv- Histological Studies

Pieces of liver was quickly removed and fixed in 10% neutral buffered formalin for histological investigations. Then sections (7 μm) were mounted on slides and dried overnight at 37°C . Sections were dewaxed in xylene and hydrated in a graded series of alcohols and stained with Harris hematoxylin and eosin according to Drury and Wallington[28]. For electron microscope small pieces fixed in 2.5% glutaraldehyde in cacodylate buffer, then washed in cacodylate buffer (0.1M, pH 7.2) and post fixed in 1% osmium tetroxide. Then placed in propylene oxide for 60 minutes, then in pure Epon 812 and incubated in a special polymerization incubator. Semithin section were cut at 1 μ and stained with toluidine blue and examined for localization of the desired tissue and accordingly ultrathin sections were prepared. Sections were stained with uranyl acetate and lead citrate[29] and examined under transmission electron microscope (Jeol, 100CXII) operated at 80 KV, Electron Microscope Center of Assiut University).

v- Statistical analysis

The results were analyzed by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test as post- Test by using Prism program for windows, version 3.0 (Graph pad softwarwe, Inc., San Diego CA. USA). The significance difference between groups was accepted at $P < 0.05$.

3. Results

The final body weights were lower in diabetic rabbits than in the control group (Table 1). The initial body weights (not shown) were similar in control and diabetic groups. The final body weights in α -lipoic acid and N-acetyl cysteine treated diabetic rabbits were higher than in the untreated diabetic group, but lower than control group. The relative weight of liver was increased in diabetic rabbits compared to the control group, however return to near nor-

mal in α -lipoic acid and N-acetyl cysteine groups. Plasma glucose concentration and ALT activity were increased significantly in diabetic rabbits compared with the control group, and were significantly decreased by α -lipoic acid and N-acetyl cysteine treatment (Table 1).

Hepatic LPO, NO, CP and DNA fragmentation % and plasma LPO and NO were significantly higher in diabetic rabbits compared with control group (Table 2). These increases were decline by α -lipoic acid and N-acetyl cysteine treatment ($P < 0.05$ vs. untreated diabetic groups).

Table 1. % of change in rabbits weight and relative weight of liver and Mean Values \pm S.E.M. of plasma glucose and ALT activity of control and different treated rabbits

Group	% of change in Rabbits weight	% of change in relative weight of liver	Plasma glucose (mg/dl)	Plasma ALT activity (U/L)
G1: Control	37.28	2.95	120.44 \pm 7.25	23.78 \pm 1.99
G2: Diabetes	-17.73	4.39	351.20 \pm 37.88 ^{a***}	38.36 \pm 2.97 ^{a***}
G3: Diabetes+ α -lipoic acid	11.98	3.16	148.87 \pm 20.57 ^{b***}	20.39 \pm 2.75 ^{b***}
G4: Diabetes+ N-acetyl cysteine	21.22	3.04	129.20 \pm 16.17 ^{b***}	14.87 \pm 1.05 ^{b***}

a= significance difference between normal control group and diabetic group.

b = significance difference between diabetic group and α -lipoic acid and N-acetyl cysteine and treated groups. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Table 2. Mean Values \pm S.E.M. of Lipid peroxidation (LPO), nitric oxide (NO), Carbonyl proteins (CP) levels and DNA fragmentation % in the plasma and liver of control and different treated rabbits

Biomarker	LPO (nmol MDA/mg protein)		NO (nmol nitrite/mg protein)		CP (nmol/mg protein)	DNA Fragmentation %
	Plasma	Liver	Plasma	Liver	Liver	Liver
G1: Control	0.18 \pm 0.02	0.55 \pm 0.4	0.82 \pm 0.1	4.02 \pm 0.36	13.21 \pm 1.52	25.65 \pm 3.17
G2: Diabetes	0.53 \pm 0.08 ^{a***}	1.43 \pm 0.07 ^{a***}	1.49 \pm 0.09 ^{**}	6.04 \pm 0.61 [*]	20.59 \pm 2.32 [*]	39.32 \pm 3.21 ^{a*}
G3: Diabetes+ α -lipoic acid	0.28 \pm 0.04 ^{b**}	1.06 \pm 0.17 ^{b*}	0.82 \pm 0.11 ^{b**}	4.61 \pm 0.46 ^{b*}	14.09 \pm 2.01 ^{b*}	24.01 \pm 4.31 ^{b*}
G4: Diabetes+ N-acetylcysteine	0.31 \pm 0.05 ^{b**}	1.06 \pm 0.08 ^{b*}	1.10 \pm 0.05 ^{b*}	4.41 \pm 0.33 ^{b*}	13.43 \pm 1.49 ^{b*}	28.71 \pm 3.70 ^{b*}

a= significance difference between normal control group and diabetic group.

b = significance difference between diabetic group and α -lipoic acid and N-acetyl cysteine treated groups. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Table 3. Mean Values \pm S.E.M. of glutathione (GSH) and vitamin E (Vit E) levels in the plasma and liver of control and different treated rabbits.

Biomarker	GSH (μ g /mg protein)		Vit E (μ g/mg protein)	
	Plasma	Liver	Plasma	Liver
G1: Control	0.32 \pm 0.12	2.34 \pm 0.18	1.19 \pm 0.22	5.20 \pm 0.51
G2: Diabetes	1.34 \pm 0.12 ^{a***}	1.52 \pm 0.11 ^{a*}	0.44 \pm 0.04 ^{a**}	2.39 \pm 0.33 ^{a***}
G3: Diabetes+ α -lipoic acid	0.68 \pm 0.13 ^{b*}	2.46 \pm 0.43 ^{b*}	1.09 \pm 0.22 ^{b*}	6.09 \pm 0.22 ^{b***}
G4: Diabetes+ N-acetylcysteine	0.86 \pm 0.11 ^{b*}	2.68 \pm 0.17 ^{b*}	1.87 \pm 0.12 ^{b***}	4.40 \pm 0.42 ^{b***}

a= significance difference between normal control group and diabetic group.

b = significance difference between diabetic group and α -lipoic acid and N-acetyl cysteine and treated groups. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Table 4. Mean Values \pm S.E.M. of Catalase (CAT), Superoxide dismutase (SOD), Glutathione reductase (GR), Glutathione peroxidase (GPx), Glutathione S-Transferase (GST), and γ -glutamyl transpeptidase (GGT) activities in the plasma and liver of control and different treated rabbits

Biomarkers		G1 Control	G2 Diabetes	G3 Diabetes+ α -lipoic acid	G4 Diabetes+ N-acetylcysteine
CAT (U/min/mg protein)	Plasma	0.82 \pm 0.22	1.65 \pm 0.21 ^{a*}	1.03 \pm 0.24 ^{b*}	0.88 \pm 0.14 ^{b*}
	Liver	92.28 \pm 9.81	131.99 \pm 13.88 ^{a*}	90.14 \pm 1.36 ^{b*}	66.79 \pm 8.07 ^{b**}
SOD (U/min/mg protein)	Plasma	0.28 \pm 0.04	0.15 \pm 0.02 ^{a*}	0.28 \pm 0.05 ^{b*}	0.30 \pm 0.03 ^{b*}
	Liver	4.42 \pm 0.76	2.57 \pm 0.31 ^{a*}	4.81 \pm 0.78 ^{b*}	4.96 \pm 0.39 ^{b*}
GR (U/min/mg protein)	Plasma	0.08 \pm 0.01	0.11 \pm 0.01 ^{a*}	0.08 \pm 0.01 ^{b*}	0.08 \pm 0.01 ^{b**}
	Liver	1.33 \pm 0.16	1.86 \pm 0.11 ^{a*}	1.29 \pm 0.15 ^{b*}	1.07 \pm 0.16 ^{b**}
GPx (U/min/mg protein)	Plasma	0.09 \pm 0.02	0.16 \pm 0.01 ^{a**}	0.12 \pm 0.01 ^{b*}	0.10 \pm 0.01 ^{b**}
	Liver	0.33 \pm 0.06	1.01 \pm 0.08 ^{a***}	0.81 \pm 0.04 ^{b*}	0.64 \pm 0.07 ^{b**}
GST (nmol/min/mgprotein)	Plasma	4.11 \pm 0.83	6.44 \pm 0.21 ^{a*}	4.87 \pm 0.53	5.85 \pm 0.56
	Liver	37.39 \pm 3.21	49.22 \pm 2.37 ^{a*}	39.82 \pm 2.97 ^{b*}	37.02 \pm 3.23 ^{b*}
GGT (nmol/min/mgprotein)	Plasma	0.66 \pm 0.08	0.37 \pm 0.05 ^{a*}	0.80 \pm 0.13 ^{b*}	0.74 \pm 0.11 ^{b*}
	Liver	22.41 \pm 1.181	16.21 \pm 1.03 ^{a*}	26.84 \pm 1.76 ^{b***}	21.31 \pm 1.76 ^{b*}

a= significance difference between normal control group and diabetic group.

b = significance difference between diabetic group and α -lipoic acid and N-acetyl cysteine and treated groups. * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

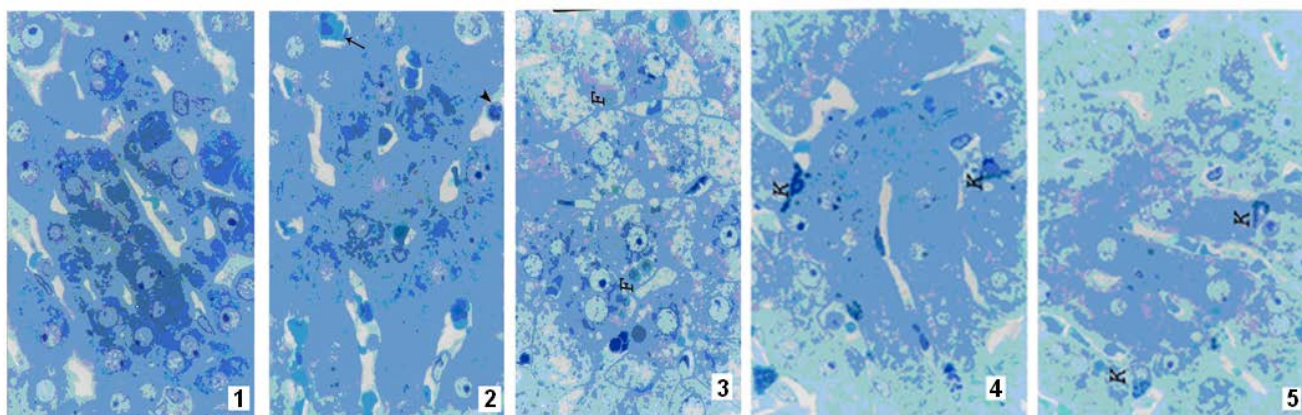


Figure 1-5.

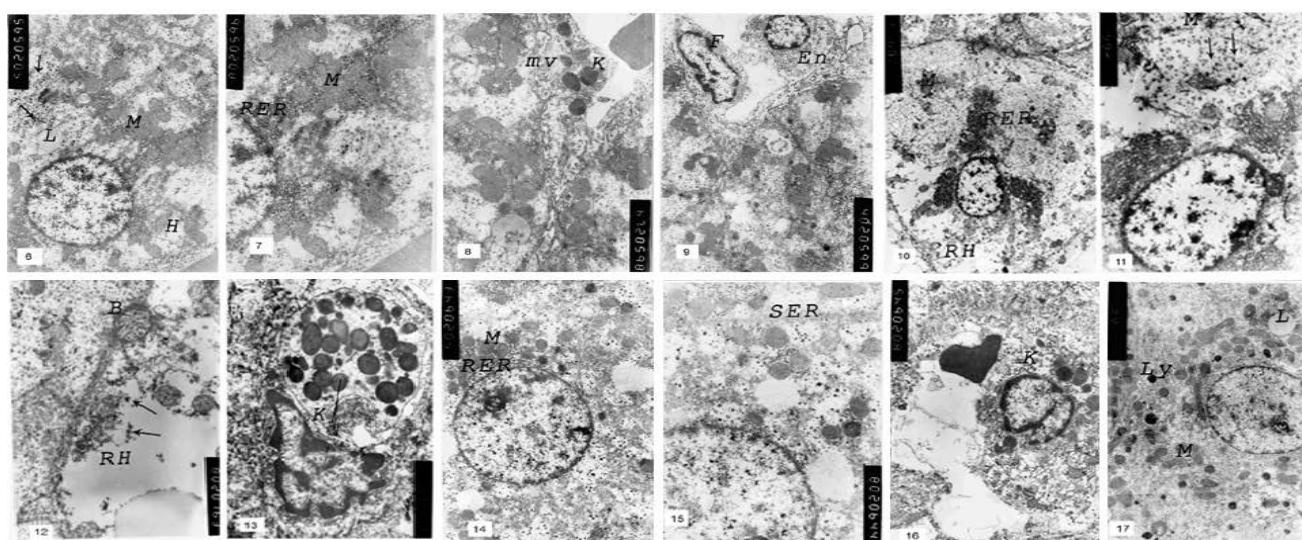


Figure 6-17.

Hepatic GSH concentration was significantly decreased (34%) in diabetic rabbits compared with the control group (Table 3), and this decrease was overcorrected by α -lipoic acid (61%) and N-acetyl cysteine (70%) versus untreated diabetic group. However, in plasma GSH was significantly increased (318%) in diabetic rabbits compared with the control group, and this increase was reduced by α -lipoic acid (49%) and N-acetyl cysteine (41%) versus untreated diabetic rabbits. Vit E concentration was significantly increased in plasma ($P < 0.01$) and in hepatic tissue ($P < 0.001$) compared with the control group (Table 3). This decreased was overcorrected in plasma and tissue by α -lipoic acid and N-acetyl cysteine treatment (145% and 309% respectively) and in hepatic tissue by α -lipoic acid treatment (150%) versus untreated diabetic rabbits.

CAT, GR, GPx and GST activities were significantly increased ($P < 0.05$), however, SOD and GGT activities were decreased ($P < 0.05$) in plasma and hepatic tissues of diabetic rabbits compared with the control group (Table 4). The previous alternation in the enzymatic antioxidant activities were overcorrected by α -lipoic acid and N-acetyl cysteine treatment versus untreated diabetic rabbits.

Examination of semithin sections in the liver of control

rabbits showed that the hepatic cell plates are radiating from central vein. Blood sinusoids separate the plates from each others with uniform distribution of cell organelles (Fig. 1). After alloxan treatment, dilated and congested blood sinusoids were observed (Fig. 2). Hydropic degenerative changes were observed in the hepatocytes with multiple fat droplets. The cellular organelles are densely packed against a background of less densely stained cytoplasmic matrix (Fig. 3). After α -lipoic acid treatment, an improvement in the dilatation and congestion of the blood sinusoids with multiple observed Kupffer cells (Fig. 4) was shown. However, after N-acetyl cysteine treatment, normal architecture of liver was observed apart from excess Kupffer cells in comparison to control (Fig. 5).

Semithin section image of normal rabbits liver showing hepatic cell plates radiating from central vein. Blood sinusoids separate the plate from each others. Note the uniform distribution of cell organelles (Fig1), congested blood sinusoids. Note the RBCs (arrow) and WBCs (arrow head) in the sinusoids (Fig2), hydropic degenerative changes in the hepatocytes with multiple fat droplets (F), cellular organelles are densely packed against a background of less densely stained cytoplasmic matrix (Fig 3). The liver of

diabetic rabbits treated with α -lipoic acid showing an improvement in the dilatation and congestion of the blood sinusoids with multiple observed Kupffer cells (K) (Fig 4) and the liver of diabetic rabbits treated with N-acetylcysteine showing normal architecture apart from excess Kupffer cells (K) in comparison to control (Fig5). (Toluidine blue X1000)

The hepatocyte of control rabbits appears with characteristic rounded euchromatic nucleus and uniformly distributed organelles in the cytoplasm. Glycogen rosettes and lipid droplets are observed. Some areas of hyaloplasm are visible (Figs. 6&7). Microvilli are observed between the surfaces of two hepatocytes and abutting on the perisinusoidal space whereas the Kupffer cell and fat storing cell are present. Blood sinusoid with endothelial lining is observed (Figs. 8&9). After alloxan treatment, a small sized nucleus of hepatocyte with a perinuclear aggregation of RER cisternae, however it is not observed in the peripheral cytoplasm. The number of mitochondria in the hepatocyte was reduced with wide areas of rarefied hyaloplasm (Fig. 10). A large amount of glycogen rosettes and multiple microbodies were clearly observed in some hepatocytes (Fig. 11). Other hepatocytes showed wide part of cytoplasm largely devoid of organelles and amorphous material could be detected within the rarefied hyaloplasm (Fig. 12). Kupffer cell was present in the perisinusoidal space with irregular heterochromatic nucleus and large number of phagosomes that were surrounded by a well delineated membrane (Fig. 13). After α -lipoic acid treatment, an improvement in the amount of mitochondria and RER, their distribution was more or less similar to those in the control. Also large amount of glycogen rosettes and multiple SER were detected (Figs. 14&15). Kupffer cell with irregular heterochromatic nucleus and few phagosomes was observed in the perisinusoidal space (Fig. 16). After N-acetyl cysteine treatment, the hepatocytes appeared more or less similar to control (Fig. 17).

Electron micrograph of the liver of normal rabbits showing uniformly distributed mitochondria in the cytoplasm. Some areas of hyaloplasm (H) are visible. Mitochondria (M), RER, glycogen rosettes (arrow) and lipid droplets (L) are observed (Fig6 X5000). A magnified part of the previous figure showing groups of mitochondria separated by RER cisternae (Fig7 X8000) Also, in the liver of normal rats, Microvilli (mv) are observed between the surfaces of two hepatocytes and abutting on the perisinusoidal space and Kupffer cell (K) in that space (Fig8 X6700), Fat storing cell (F) in the perisinusoidal space and endothelial lining of capillary (En) (Fig9 X8000). Electron micrograph of the liver of diabetic rabbits showing a small sized nucleus of hepatocyte with a perinuclear aggregation of RER cisternae while it is not observed in the peripheral cytoplasm. Number of mitochondria was reduced in the hepatocyte with wide areas of rarefied hyaloplasm (RH) (Fig10 X2700). A large amount of glycogen rosettes (arrow) and multiple microbodies (M) (Fig11 X6700). A wide part of cytoplasm was largely devoid of organelles, amorphous material (ar-

row) within the rarefied hyaloplasm (RH) and bile canaliculi (B) were observed between the surfaces of two hepatocytes (Fig12 X8000). Kupffer cell (K) with irregular heterochromatic nucleus and containing a large number of phagosomes that were surrounded by a well delineated membrane (Fig13 X10000). In diabetic rabbits treated with α -lipoic acid liver showed an improvement in the amount of mitochondria (M) and RER, their distribution was more or less similar to those in the control (Fig14 X5000), Large amount of glycogen rosettes and multiple SER (Fig15 X8000), Kupffer cell (K) with irregular heterochromatic nucleus and containing few phagosomes (Fig16 X8000). And in diabetic rabbits treated with N-acetylcysteine liver showed the hepatocyte appeared more or less similar to control. Lipid (L), lysosomes (Ly) and mitochondria (M) were observed (Fig17 X4000).

4. Discussion

The present results of plasma glucose and ALT activity in alloxan-induced diabetic rabbits (Table 1) are consistent with the finding in rats[30&31], in mice[32] and in rabbits[33]. The increment of ALT activity may be mainly due to the leakage of this enzyme from the hepatocyte cytosol into the blood stream[34], which gives an indication on the hepatotoxic effect of alloxan. Treatment of diabetic rabbits with α -lipoic acid or N-acetyl cysteine for 7 days restores the above parameters to their normal levels. However, myrtle oil which has anti-hyperglycemic effect failed to restore the elevated ALT activity in alloxan diabetic rabbits[35]. In the present work rabbits treated with alloxan after 4 weeks showed marked weight loss and increased relative liver weight. These results are consistent with previous reports in alloxan-induced diabetes in rabbits[36] and in rats[37]. The increase in liver weight is possibly due to the infiltration of the organ by fatty tissue[38]. Ectopic fat depositions in peripheral tissue can precede diabetes and could occur with the development of glucose intolerance and insulin resistance[39].

Islet cell death during the development of diabetes by alloxan is thought to be mediated by ROS. Alloxan is selectively accumulated in β cells through uptake via the glucose transporter in the plasma membrane. In the presence of GSH alloxan generates ROS in a cyclic reaction between GSH and its reduction product, dialuric acid[12& 40]. The highly reactive hydroxyl radical, which is one of the ROS generated in this process, is considered to be responsible for the formation of carbonyl groups in protein leading to loss of thiol groups[41&42]. The present results together with previous findings (40&43) indicate that ROS generated by alloxan contributes to the β -cell toxicity and hyperglycemia. α -lipoic acid or N-acetyl cysteine treatment significantly reduced hyperglycemia in diabetic group, by a mechanism that is independent on insulin, because antioxidant treatments were started after β -cell damage. In this point, Odetti *et al.* [44] reported that any protection of pancreatic β -cells

against STZ by the antioxidants is considered improbable. Moreover, α -lipoic acid increase glucose uptake through recruitment of the glucose transporter-4 to plasma membrane with insulin-stimulated glucose uptake[45& 46]. Similarly, N-acetyl cysteine treatment increased glucose uptake in individuals exposed to high glucose[9].

Apoptosis can be considered as a finely regulated mechanism to maintain the genome stability of multicellular organisms by eliminating cells with severe DNA damage caused by oxidative stress[47]. DNA fragmentation is considered as an indication of apoptosis[48]. In the present study, alloxan elevated significantly DNA fragmentation in liver (Table 2). In this point, hyperglycemia induced by glucose administration for few hours leads to DNA fragmentation similar to apoptosis in some organs of rats[49]. The interpretation of the increased DNA fragmentation may be due to hyperglycemia, which increased endogenous glyceraldehydes and H_2O_2 levels, which have strongest ability to damage DNA[50&51]. In the present study, α -lipoic acid or N-acetyl cysteine significantly counteracted hyperglycemia and elevated DNA fragmentation (Table 2). α -lipoic acid decreases DNA fragmentation in alloxan-induced diabetic rabbits through the enhancement of glucose uptake resulting in normoglycemia and scavenging intracellular superoxides improving mitochondrial function and DNA damage[9]. Moreover, N-acetyl cysteine has the ability to reduce apoptosis in kidney[44]. Extracellular thiols may be important for the inhibition of apoptosis because DNA fragmentation was inhibited by physiologically low levels of L-cysteine[52].

ROS metabolism is disrupted in diabetes and animal models of diabetes[53&54]. Oxidative stress biomarkers are indicative of damages to carbohydrates, lipids and proteins by ROS[54]. It has been established in mammalian system that direct damage to proteins or chemical modification of amino acids in proteins during oxidative stress can give rise to protein carbonyls[55]. In the present study, CP was significantly increased in hepatic tissues of diabetic rabbits (Table. 2). Elevation of carbonyl protein in hepatic tissue of diabetic rabbits was reported by[36]. The significance of hepatic complications in diabetes may rise due to the glycosylation of protein resulting in numerous alterations in hepatic ultra structures as seen in the present study[56]. The present data showed that treatment of diabetic rabbits with α -lipoic acid or N-acetyl cysteine significantly reduced CP. In consistence with these data Bitar *et al.*[57] found that α -lipoic acid ameliorated the diabetes related defects in glucose metabolism, protein oxidation as well as the activation by insulin of the various steps of the insulin signaling pathway. The ability of α -lipoic acid or N-acetyl cysteine to reduce CP may be mediated by reduced hyperglycemia and oxidative stress through the recycling of reduced Vit C and GSH[58&59].

Irreversible cell damage occurs when the cell is no longer able to maintain its GSH content[36]. Therefore, measurement of GSH in biological samples is important for the understanding of GSH homeostasis in disease[60]. In the pre-

sent study, a significant decrease of GSH level was observed in hepatic tissue (Table. 3), which is in agreement with other authors[61&62]. In this point, STZ-injection caused a rapid decrease in the GSH content of liver and pancreatic islets, however, the fall in islets was more pronounced and persistent[63]. The decrease in GSH content is probably due to ROS generation by alloxan and hyperglycemia[64]. In addition, GSH is extruded from cells undergoing apoptosis[65]. This finding is in harmony with the present results where GSH content is increased in plasma of diabetic rabbits (Table. 3). In the present work both α -lipoic acid or N-acetyl cysteine restored the depleted GSH in diabetic rabbits (Table. 3). Similar finding was reported[9&66] they found that α -lipoic acid or N-acetyl cysteine increases GSH. Lately, α -lipoic acid or N-acetyl cysteine was reported to prevent disturbances in GSH homeostasis in blood and liver in alloxan-diabetic rabbits[67]. The increased GSH content in hepatic tissues of diabetic rabbits treated with N-acetyl cysteine is due its role as a precursor of cysteine, which in turn enters in the GSH synthesis cycle[68].

The key indicator of GSH metabolism in an organ is the GGT, which initiates the catabolism of GSH by removing the γ -glutamyl group from GSH[69]. The present data showed a significant reduction in hepatic GGT activity of diabetic rabbits (Table. 4). The inhibition of GGT activity may lead to the inability of tissue to recover the constituent amino acids of GSH[[70]. So, the increased GGT activity that are observed in diabetic rabbits treated with α -lipoic acid or N-acetyl cysteine probably represents harmonizing mechanism to recover GSH to about its normal control level (Table. 4).

The reaction of ROS with membrane lipoproteins produces malondialdehyde[71]. The present data revealed that persistent hyperglycemia through alloxan treatment generated ROS attack produced a significant increase in LPO in hepatic tissue and plasma of diabetic rabbits than control (Table 2). Previous studies done on diabetic animals showed the same results[36&72]. Elevated level of LPO is one of the characteristic features of chronic diabetes[73]. In diabetes fatty acid oxidation causing a marked proliferation of peroxisomes in hepatocytes that ultimately could be associated with the development of hepatocellular tumours[74]. In the present study, the administration of α -lipoic acid or N-acetyl cysteine to diabetic rabbits caused marked decrease in LPO level (Table 2). In this aspect, α -lipoic acid inhibit LPO in the heart and skeletal muscle mitochondria of aged rats[75]. Because it has an effective role as a scavenger of lipoperoxides at their mitochondrial source and preventing LPO[76]. Also, N-acetyl cysteine has a protective effect on pulmonary LPO[77].

GPx protect cells against LPO and shares the substrate, H_2O_2 with catalase[78]. It has been observed that GPx can be rapidly induced when cells are exposed to oxidative stress[79]. In agreement with other reports, the present study showed that the activity of CAT and GPx was higher in diabetic rabbits than normal[36&80]. The selective cytotoxicity of alloxan to the β cell results from the coincidence

of two features: a rapid cellular uptake of the drug and an exquisite sensitivity to peroxide itself attributable to low peroxidase activity (GPx and CAT)[2]. Plasma GPx activity has been elevated in both types of diabetes[81]. The increased GPx activity is a consequence of increase in the pentose-phosphate pathway in the diabetic animals, because GPx utilizes GSH in the elimination of H_2O_2 . Oxidized GSH thus formed requires NADPH for its subsequent reduction by GPx[82]. In the present study, it was found that α -lipoic acid or N-acetyl cysteine administration normalize the GPx and catalase in hepatic tissue and plasma of diabetic rabbits (Table 4). In this aspect, restoration of both CAT and GSH in vonadate-treated diabetic rats were observed[83]. Moreover, vandate therapy could effectively normalize the GPx in diabetic rat[84].

Superoxide dismutase protects the organism against the deleterious effects triggered by the superoxide radical[82]. The present study showed a significant decrease in SOD activity in hepatic tissue and plasma of diabetic rabbits. This finding is in accordance with those previously described for SOD activity in the liver, kidney, spleen, heart, testis, pancreas, skeletal muscle and erythrocytes in alloxan or STZ induced diabetes in rats (85, 86 & 87). The reduction in SOD activity may be due to the direct damaging effect of free radicals on the enzyme[88]. In this respect, SOD treatment can protect *in vivo* and/or *in vitro* against the high toxic potential of the superoxide radicals in alloxan-induced diabetic rats[89&90]. α -lipoic acid or N-acetyl cysteine administration to diabetic rabbits restored the SOD activity to that of normal control. Similar result was obtained in intestine and adrenal cortex by treatment of STZ-induced diabetes in rats with insulin or GSH[91]. In addition, SOD acts prophylactically against alloxan-induced diabetes in mice[89].

Additionally, activation of polyol pathway by hyperglycemia can diminish NADPH available for GR and may cause a significant decrease in GSH level[36]. In the present study, GR activity was increased in hepatic tissue and plasma of diabetic rabbits. GR activity was reduced after 3 weeks in liver of STZ-treated rats[92]. In contrary, GR activity not changed in hepatic tissue of rat which suggested that GR may not be involved in protection against STZ-induced diabetes[84&93]. GST is localized in hepatocytes and in proximal tubule cells, which are the main cells responsible for the most rapid GSH turnover in liver. In the present study, alloxan-induced diabetes in rabbits caused a significant increase in the GST activity in hepatic tissue and plasma compared to that of normal control. The increase in GST activity indicates the apparent stress in terms of increased concentration of cellular metabolites[94].

In the present study, the decreased Vit E level in hepatic tissue and plasma of diabetic rabbits compared to that of the control animals was observed (Table 3). Hepatic Vit E was decreased in rats with STZ-induced diabetes of 20 weeks duration[95]. Deficiency of Vit E may result from increased its consumption due to increased oxidative stress associated with hyperglycemia[96]. The demand for the Vit E in dia-

betes is increased[97]. Currently, the molecular and cellular effects of Vit E have been explained either by acting as a mere antioxidant preventing damage to membranes and regulating their activity by specifically scavenging ROS[98], or by interacting and regulating specific enzymes and transcription factors influencing cellular structures[99]. In the present study α -lipoic acid or N-acetyl cysteine treatment of diabetic rabbits restored the Vit E content to that of normal control. It has been observed that α -lipoic acid prevented the consequences of Vit E deficiency in rats[100], due to its ability to react with free radicals which are prooxidants for Vit C and E[66]. Vit E primarily being oxidized to tocopheroxyl radical and then being reduced back to tocopherol by GSH[101]. So, treatment of the diabetic rabbits with N-acetyl cysteine restored the Vit E content to that of normal control. Combination of Vit E and α -lipoic acid may provide protection against apoptosis[102].

NO is a multifunctional biomolecule involved in a variety of physiological and pathological processes. A large amount of NO is toxic to the host and causes several diseases such as apoptosis and diabetes[103]. The present study showed that NO was significantly increased in hepatic tissue and plasma of diabetic rabbits (Table 2). NO synthase dependent reactivity was impaired in blood vessels during diabetes[104]. Endothelium dependent relaxation was enhanced after STZ-induced diabetes in renal artery[105]. NO synthase activity is a pathogenic factor in diabetes development. So, administration of NO synthase inhibitor largely suppressed the development of hyperglycemia following STZ treatment in mice[106]. Moreover, NO mediates the inhibitory effects of cytokines on glucose stimulated insulin secretion by human islets[107]. During the development of experimental diabetes the inhibitory serotonergic effect may be mediated by the NO pathway[108]. In the present study, the level of NO was reverted back to near normal in liver and plasma by treatment of diabetic rabbits with α -lipoic acid or N-acetyl cysteine. α -lipoic acid inhibits NO synthesis during endotoxemia in rats by down regulating NOS expression[109]. N-acetyl cysteine has a protective effect by reducing iNOS expression via diminishing LPO[110]. Moreover, N-acetyl cysteine might have protective actions in chronic inflammatory liver disease, mediated partially through the modulation of NO production[111].

The observed degenerative changes in the hepatocytes might occur as a result of glycated effected of hyperglycemia on the proteins thus explained the diabetic changes in the subcellular organelles of liver. These results are in accordance with the work of Myint *et al.*[112] who found that in diabetic conditions liver proteins are more susceptible to glycation than other tissue proteins. However, the inducing effects of hyperglycemia on ROS as well as LPO result in cellular damage as well as apoptotic changes. N-acetyl cysteine treatment improved dilatation in the blood capillaries as well as reduced aggregation of Kupffer cells that were observed in excess in diabetic animals. Kupffer cells may have a role in the removal of debris in degenerated tissues

and may play a role in the production of ROS after their activation. In rheumatoid arthritis, the production of ROS from activated macrophages are inhibited by the gold (I)-containing compounds that induce cellular antioxidative stress genes [113&114]. In the present study, the observed reduction in Kupffer cells following the administration of α -lipoic acid or N-acetyl cysteine might confirm the potential role of these activated cells in the manifestation of oxidative stress in diabetic rabbits. In conclusion: oxidative stress in alloxan induced diabetic rabbits plays a crucial role in hepatic degenerative changes. Treatment of diabetic rabbits with α -lipoic acid or N-acetyl cysteine caused normoglycemia and liver cell reprogramming due to its antioxidant activities. Beneficial action seems to result mainly from direct scavenging of ROS and restoring GSH redox state.

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