www.nephropathol.com DOI: 10.15171/jnp.2019.06 J Nephropathol. 2019;8(1):e06

Journal of Nephropathology

Effect of alpha-lipoic acid on antioxidant gene expression and kidney injury in alloxan-induced diabetic rats

Parisa Jamor[1], Hassan Ahmadvand[2,3*], Hesam Ashoory[2], Esmaeel Babaeenezhad[1,4]

[1]Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
[2]Razi Herbal Researches Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[3]Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[4]Student of Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 14 February 2018
Accepted: 10 June 2018
Published online: 29 June 2018

Keywords:
Diabetes mellitus
Alpha-lipoic acid
Glutathione peroxidase
Catalase

ABSTRACT

Background: Myeloperoxidase (MPO) is involved in the initiation, progression, and complications of atherosclerosis in diabetic patients.

Objectives: In the current study, the impact of alpha-lipoic acid (LA), a natural antioxidant and a cofactor in the enzyme complexes on MPO, catalase (CAT) and glutathione peroxidase (GPx) activity, glutathione (GSH) and malondialdehyde (MDA) level, histopathology of kidney and expression of antioxidant enzymes, superoxide dismutase (SOD), GPx and CAT which are involved in the detoxification of reactive oxygen species (ROS), was evaluated in alloxan-induced diabetic rats.

Materials and Methods: In this study, 30 male Rattus norvegicus rats randomly divided into three groups; control (C), non-treated diabetic (NTD), and LA-treated diabetics (LATD) was induced by alloxan monohydrate (100 mg/kg; subcutaneous [SC]). Then treatment was performed with alpha-LA (100 mg/kg intraperitoneal (i.p) daily to 6 weeks). Blood sample of animals collected to measure levels of MPO, CAT and GPx activity GSH and MDA. Kidney paraffin sections were prepared to estimate histological studies and to measure quantitative gene expression SOD, GPX and CAT in kidney.

Results: Induction of diabetes led to a significant increase in MPO and MDA, reduced GSH level and GPx and CAT activities ($P < 0.05$). However, treatment with alpha-LA led to a significant elevation in GPx, CAT and GSH levels with a reduction in MPO activities and MDA levels ($P < 0.05$). Furthermore, the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis results showed increased expressions of GPx, CAT and SOD enzyme in the treatment group compared with the diabetic control group. Histopathological lesions such as increased glomerular volume and lymphocyte infiltration were attenuated in the alpha-LA treated group.

Conclusions: Our findings indicated that alpha-LA supplementation is effective in preventing complications induced by oxidative stress and atherosclerosis in diabetic rats.

Original Article

Implication for health policy/practice/research/medical education:
Alpha-lipoic acid supplementation is effective in preventing complications induced by oxidative stress and atherosclerosis in diabetic rats.

Please cite this paper as: Jamor P, Ahmadvand H, Ashoory H, Babaeenezhad E. Effect of alpha-lipoic acid on antioxidant gene expression and kidney injury in alloxan-induced diabetic rats. J Nephropathol. 2019;8(1):e06. DOI: 10.15171/jnp.2019.06.

1. Background

Diabetes is considered as an important chronic disease and a leading cause of death and disability worldwide. It is a metabolic disorder recognized by hyperglycemia and incomplete secretion or action of endogenous insulin (1). There have been 171 million cases of diabetes in 2000 and the number will jump to 366 million in 2030 (1). The disease is accompanied by numerous implications

*Corresponding author: Prof. Hassan Ahmadvand, Ph.D.;
Email: hassan_a46@yahoo.com

affecting different organs such as the eyes, kidneys, nerves, heart, and blood vessels (atherosclerosis) (2). Oxidative stress is characterized by excessive production of free radicals and defect in body antioxidant defense (2, 3). Several studies have found that oxidative stress caused by reactive oxygen or nitrogen species following hyperglycemia play a significant role in the pathogenesis of diseases. Diabetes show increased levels of plasma thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide and lipoperoxidase (3). Recent studies have shown abnormal lipid peroxidation in diabetes. Therefore diabetes condition is associated with increased production of free radicals and lipid peroxidation that caused tissue damage and oxidative stress (4). Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) antioxidant enzymes are major components of body defense mechanism to neutralize these free radicals (5). Free radicals are produced by a variety of mechanisms such as auto-oxidation of glucose (6), glycated proteins, and glycation of anti-oxidative enzymes, which limit their capacity to scavenge free radicals (4, 5). Weak defense system of the body is unable to counteract the enhanced reactive oxygen species (ROS) generation results in organ damages. Glutathione (GSH) and malondialdehyde (MDA) level as a lipid peroxidation markers are affected by diabetes mellitus. There is evidence that oxidative stress caused by diabetes condition plays an important role in endothelial dysfunction which finally led to atherogenesis and cardiovascular implications. Augmented production of superoxide anion is found to have a major role in this process (7). Myeloperoxidase (MPO) is a hemoprotein pro-oxidant enzyme present in the granules of leukocytes. Besides its potent bactericidal activity by ROS production, MPO is known to be a marker of endothelial dysfunction and atherosclerosis, exerting its effects through different mechanisms (8). Furthermore, diabetes-induced oxidative stress causes diabetic nephropathy (DN) which increases the relative risk of cardiovascular diseases 10-fold higher compared to diabetic patients without nephropathy. DN is characterized by structural changes such as thickening of the basement membranes, mesangial sclerosis, and arteriolar hyalinosis (9). Several antioxidants such as vitamins A, C, and E, mixed carotenoids, α-lipoic acid (LA), coenzyme Q10 and quercetin (10) have been investigated for their potential protective role in preventing oxidative stress in diabetic patients.

Alpha-LA is a naturally occurring compound that is produced endogenously by plants and animals, acting as a cofactor for enzyme complexes such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Also LA acts as an antioxidant in both oxidized (LA) and reduced (dihydrolipoic acid; DHLA) forms (11). Beside its role in direct radical scavenging and metal chelating activity, there are reports that LA may act indirectly to maintain the cellular antioxidant status by regeneration reduced forms of essential intracellular antioxidants such as α-tocopherol and CAT (12).

2. Objectives

The present study was conducted to investigate the effectiveness of LA supplementation on antioxidant profile, gene expression and histopathological changes in the kidney of alloxan-induced diabetic rats and to evaluate its beneficial effect for treatment of diabetes mellitus.

3. Materials and Methods

3.1. Experimental design

Thirty adult male Wistar rats (*Rattus norvegicus*) (180 ± 20 g) were purchased from the animal house of the Shahid Beheshti Medical University, Tehran, Iran. The animals were transferred to animal laboratory of Razi Institute, Lorestan, Iran. They were housed in cages under standard laboratory conditions with a constant 12-hour light/dark cycle. The temperature was maintained at 23 ± 2°C with 30%–55% relative humidity and fed with standard pellet feed and water *ad libitum*. The animals were randomly divided into three groups of ten; control (C), non-treated diabetic (NTD), and LA-treated diabetics (LATD). The rats made diabetics in accordance with our previous study (13). The rats were fasted for 24 hours with free access to water prior to the administration (100 mg/kg; subcutaneous [SC]) of freshly prepared alloxan monohydrate (Sigma, St. Louis, MO, USA) dissolved in ice-cold normal saline. After 2-3 days, animals having fasting blood glucose concentration ≥250 mg/dL were considered diabetic and used for the investigation. Alpha-LA powder (Sigma, St. Louis, MO, USA) was dissolved in ethanol (96%) and normal saline with 7:3 proportions for daily injection (100 mg/kg/body weight, SC) for 6 weeks.

3.2. Sample collection

After 6 weeks of treatment, animals anesthetized with ketamine (87 mg/kg i.p.; intraperitoneal) and xylazine (13 mg/kg i.p.). Blood samples were taken from the animals' hearts and the serum was separated by centrifugation (3000 rpm at 4°C for 15 minutes) to measure serum biochemical parameters. Kidney and liver organs were rinsed with isotonic saline and homogenized with phosphate-buffered saline (10%, pH 7.4) then centrifuged and the supernatant was kept at -20°C and used to measure biochemical parameters. Tissue section

was prepared from kidney and they were molded in paraffin sections to calculate the tissue parameters. Immediately after euthanization, the kidneys were frozen at -70°C to perform molecular analysis and gene expression investigation.

3.3. Biochemical analysis

Fasting blood sugar (FBS), creatinine and urea were determined using commercial kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's protocol. Serum and tissue CAT activity was determined in accordance with our previous study (14). Serum and tissue GPx content was determined in accordance with our previous study (14). Serum and tissue MPO activity was determined according to our previous study (15). The measurement is based on the oxidation of O-dianisidine dihydrochloride by hydrogen peroxide at 450 nm. Serum and tissue MDA levels were determined as our previous protocol (16). The measurement is based on the TBA 6% and TCA 1% so boiled for 30 minutes and centrifuged then read at 540 nm (14). The measurement is based on the Tris-EDTA 2M (pH = 8) and DTNB 1M then read at 540 nm. All activity assays were performed in triplicates.

3.4. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

The expression levels of antioxidant genes were investigated by real-time quantitative PCR. Briefly, 50-100 mg of frozen kidney samples of each group were added to 1 mL TriZol reagent (MO, USA) then total RNA was extracted with a chloroform solution. The micro-tube was subjected to centrifugation at 12000 rpm for 15 minutes and then isopropanol solution was used to precipitate the extracted RNA. Finally after centrifugation and washing, the RNA was dissolved in DEPC-H_2O. RNA purity and concentration were determined at 260 and 280 nm using NanoDrop spectrophotometer (Biochrom WPA Biowave II, UK). Quality of RNA was investigated by gel electrophoresis. RNA was reverse transcribed to cDNA using commercial kit according to manufacturer's instructions (Sigma kit, USA). Synthesized cDNA was treated with DNase enzyme to remove active DNA molecules. cDNA was stored at -70°C until it was used.

3.5. Real-time PCR

Real time quantitative PCR was conducted to detect the gene expression of GPx, CAT, and SOD in kidney tissue using SYBR master mix (Applied Biosystems, CA, USA) and the reaction was performed on Rotor-Gene 6000 (Corbett Research). Each 20 µL reaction volume contained 10 µL of master Mix, 1 µL of cDNA, 1 µL of each specific primers (Sangon, Shanghai, China), and 7 µL distilled water. All reactions were done in duplicate. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as a control for normalization.
Primers listed in Table 1 were designed using Primer 3 v.4.0 (http://frodo.wi.mit.edu./primer3/).

3.6. Histological evaluation

Specimens of kidney of the different groups were immediately fixed in 10% formalin. Paraffin blocks were prepared and cut to 5 µm thickness sections then stained with hematoxylin-eosin (H&E). Glomerular volume was determined as o (14) by digitalization system composed of camera and microscope (Leica ICC50 HD, Germany) at magnifications of × 40 using the formula: $VG = Area1.5 \times \beta/K$, where β is a shape coefficient of 1.38 for a sphere and K is a size distribution coefficient. Values for K vary between 1 and 1.05 for glomerular size distributions and considered to be 1.01 in the current study. For each animal, VG was calculated at least for 100 glomeruli. Lymphocyte infiltration is determined in kidney at magnifications of × 40. Lymphocytes were counted in 100 fields.

3.7. Ethical issues

All experimental protocols and steps of the tests were conducted in compliance with the regulations of the Research Ethics Committee of the university and Iranian Ethical Guidelines for the use of animals in research. Additionally all animal experiments were in accordance

Table 1. Gene-specific primer of related genes

Gene	Gene bank number	Primers position	Primers sequences(5'→3')	Product size
CAT	NC_005102.4	Forward	ATTGCCGTCCGATTCTCC	105
		Reverse	CCAGTTACCATCTTCAGTGTAG	
SOD	NC_005110.4	Forward	CGAGCATGGGTTCCATGTC	101
		Reverse	CTGGACCGCCATGTTTCTTAG	
GPX	NC_005107.4	Forward	CAGTTCGGACATCAGGAGAAT	139
		Reverse	AGAGCGGGTGAGCCTTCT	
GAPDH	NC_005103.4	Forward	ATGGAGAAGGCTGGGGCTCACCT	209
		Reverse	AGCCCTTCCACGATGCCAAAGTTGT	

with protocols approved by the United States National Institutes of Health (NIH, 1978). The research was also approved by Ethics Committee of Lorestan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Lorestan University of Medical Sciences (approval code; 21/91).

3.8. Statistical analysis

Statistical significance of different control and diabetic mice groups was assessed using SPSS 22.0. Values are expressed as mean± SD. The results obtained between groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan test. The results for histological investigations were analyzed using nonparametric Mann-Whitney U test. The differences were considered significant at $P < 0.05$. Real-time PCR results were analyzed using rest-RG software.

4. Results

4.1. Biochemical markers

The activities of enzymatic antioxidants such as CAT, and GPx in the serum, liver and kidney of control and experimental groups of rats are presented in Tables 2 and 3 respectively. The activities of CAT and GPx significantly decreased in the serum, liver and kidney of diabetic control group compared with normal control rats. The activities were restored significantly ($P < 0.05$) to normal in diabetic rats after treatment with LA. These alterations were significantly regulated by LA treatment. These data strongly suggested that LA treatment significantly enhance antioxidant capacity in diabetes.

The activities of MPO in the serum, liver and kidney of control and experimental groups of rats are represented in Table 4. The activities MPO was significantly elevated in the serum, liver and kidney of alloxan-induced diabetic rats. The activities were restored to normal in the alloxan-induced diabetic rats after treatment with LA. These alterations were significantly regulated by LA treatment.

The levels of MDA in the kidney, serum and liver and kidney of different groups of rats are represented in Table 5. The level MDA was significantly elevated in the serum, liver and kidney of alloxan-induced diabetic rats. The levels were restored to normal in the alloxan-induced diabetic rats after treatment with LA. These alterations were significantly regulated by LA treatment.

The levels of GSH in the kidney, serum and liver of different groups of rats are represented in Table 6. The level GSH was significantly reduced in the serum, liver

Table 2. Effects of alpha lipoic acid treatment on GPX activity in serum, liver and kidney

Parameter	Control	Non-treated diabetic	Lipoic acid treated-diabetic	*P* value
Serum GPX (U/mg protein)	2104±154	1806±65*	2354±111#	0.001
Liver GPX (U/mg protein)	5969±122	4513±251*	5469±646	0.26
Kidney GPX (U/mg protein)	11470±441	17460±437*	25654±474#	0.001

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

Table 3. Effects of alpha lipoic acid treatment on CAT activity in serum, liver and kidney

Parameter	Control	Non-treated diabetic	Lipoic acid treated-diabetic	*P* value
Serum CAT (U/mg protein)	568±38	437±69*	526±27#	0.002
Liver CAT (U/mg protein)	1601±38	1098±79*	1554±49#	0.008
Kidney CAT (U/mg protein)	577±10	290±72*	408±143	0.001

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

Table 4. Effects of alpha lipoic acid treatment on MPO activity in serum, liver and kidney

Parameter	Control	Non-treated diabetic	Lipoic acid treated-diabetic	*P* value
Serum MPO (U/mg protein)	75±7	149±40*	105±5#	0.003
Liver MPO (U/mg protein)	135±19	372±37*	236±28#	0.001
Kidney MPO (U/mg protein)	368±19	817±18*	480±11#	0.001

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

Table 5. Effects of alpha lipoic acid treatment on MDA activity in serum, liver and kidney

Parameter	Control	Non-treated diabetic	Lipoic acid treated-diabetic	*P* value
Serum MDA (nmol/g protein)	229.84±74.33	573.58±240.09*	329.91±112.68#	0.001
Liver MDA (nmol/g protein)	5148±422	8406±712*	5629±587#	0.001
Kidney MDA (nmol/g protein)	3044±230	5925.3±578*	4718±573#	0.001

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

and kidney of alloxan-induced diabetic rats. The levels were restored to normal in the alloxan-induced diabetic rats after treatment with LA. These alterations were significantly regulated by LA treatment.

The levels of FBS, urea and creatinine in the serum of different groups of rats are represented in Table 7. The levels of FBS, urea and creatinine were significantly elevated in the serum of alloxan-induced diabetic rats. The levels were restored to normal in the alloxan-induced diabetic rats after treatment with LA. These alterations were significantly regulated by LA treatment.

4.2. Gene expression

The expression levels of Cu-Zn SOD, Cu-Zn GPx and CAT mRNA in kidneys of all three experimental groups were assessed by RT qPCR. Compared with non-diabetic rats, Cu-Zn SOD, and GPx mRNA expression levels did not change in the kidneys of diabetic rats. Decreased levels of mRNA expression were observed in CAT. mRNA expression of all genes in the kidneys of diabetic rats was enhanced by the treatment with LA (Figure 1).

4.3. Histopathology

As illustrated in Figures 2 and 3. V_G was significantly greater in untreated diabetic rats compared with values $P<0.050$ in control rats. Treatment of diabetic rats with LA reduced V_G to normal values $P<0.050$. The V_G decreased significantly compared to the diabetic control group. Lymphocyte infiltration was significantly $P<0.050$ increase in untreated diabetic rats compared to control rats and treatment with LA significantly $P<0.050$ inhibited increases in LATD group compared to the diabetic group.

5. Discussion

The present study was conducted to investigate the antidiabetic activity of alpha-LA in alloxan-induced diabetic rats. Diabetes model was induced by alloxan which selectively destroys the insulin-producing β-cells in the pancreas. Alloxan is reduced to dialuric acid with intracellular GSH. Dialuric acid and alloxan induce the generation of ROS, causing damage to β-cells and inhibiting insulin secretion (17). The first line of body defense against free radicals is GPx, SOD and CAT enzymes. GPx which is found in the blood and cells, play an important role against peroxidative damage of lipids and nucleic acids. It is able to detoxify a wide range of peroxides. Superoxide anion ($O_2^{\cdot-}$) is considered to be most important ROS. It is dismutated by SOD to produce oxygen and hydrogen peroxidase (H_2O_2)

Table 6. Effects of alpha lipoic acid treatment on GSH activity in serum, liver and kidney

Parameter	Control	Non-treated diabetic	Lipoic acid treated-diabetic	*P* value
Serum GSH (nmol/mg protein)	105±11	77±9*	94.05±10#	0.00
Liver GSH (nmol/mg protein)	684±52	521±14*	863±97#	0.00
Kidney GSH (nmol/mg protein)	400±28	267±41*	312±73#	0.003

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

Table 7. Effects of Alpha lipoic acid treatment on FBS, urea and creatinine in serum

Parameter	FBG (mg/dL)	Serum Urea (mg/dL)	Serum creatinine (mg/dL)
Control	179.16±15.95	48.8±8.1	0.94±0.21
Non-treated diabetic	402±43.1*	105±52.1*	3.21±0.83*
Lipoic acid treated-diabetic	244±19.07#	75.8±8.4#	2.93±1.24#

Values are represented as mean ± SD; * Significant change in comparison with control at $P<0.050$. # Significant change in comparison with lipoid acid-treated diabetic at $P<0.050$.

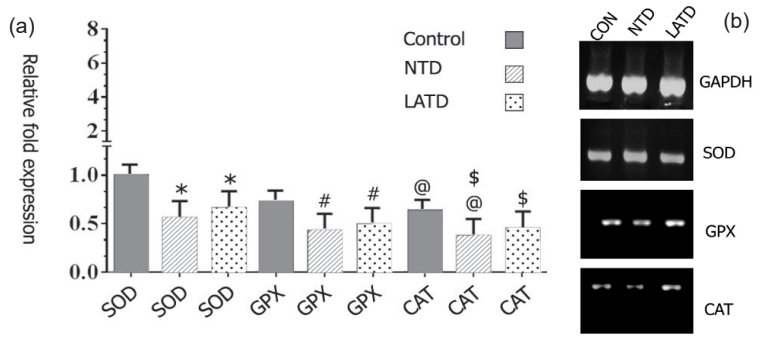

Figure 1. Quantitative expression analysis of the SOD, GPX and CAT. Comparison between all of groups (a) RT qPCR bands on agarose gel and their relative intensities are indicated in (b).
Notes: These data were applied with rest-RG software. SOD and GPX diabetic group is not different to control group but CAT is DOWN-regulated in sample group (in comparison to control group) and in CAT @ significant change in diabetic group comparison with control at $P < 0.050$ (a). Results are represented as mean ± SD
SOD, GPX and CAT are UP-regulated in treatment group (in comparison to diabetic group) at* $P < 0.05$ in SOD, # $P < 0.05$ in GPX and $ $P < 0.05$ in CAT (a). SOD, GPX and CAT obtained from groups shows the significant differences between groups. Results are represented as mean ± SD.

which finally is dismutated by CAT into water and oxygen (18). The significant reduction in the CAT and GPx activities in diabetic rats confirms the enhanced oxidative stress under diabetic condition. However, reports on the response of antioxidant enzymes in diabetes vary considerably. There are findings showing either increased, decreased or unchanged activities of antioxidant enzymes (14,19). The over-production of ROS might overwhelm endogenous antioxidant enzymes. Furthermore, $O_2^{\bullet-}$ is able to block CAT directly (20). GPx is relatively a stable enzyme, but some findings suggest the enzyme is deactivated in excessive production of free radicals. LA treatment significantly increased the activity of antioxidant agent in treatment group indicating its protective role in oxidative stress. LA is active in both hydrophobic and hydrophilic environments which make it a strong agent to exert its antioxidant effects in cytosol, cell membrane and in serum. It accelerates the regeneration of antioxidant enzymes by reducing their oxidized form (21). High blood sugar brings glucose auto-oxidation, lipid peroxidation, increasing the formation of arachidonic acid and MDA (22). TBARB level measurement is a way of revealing the peroxidative damages as one of the markers of lipid peroxidation which is caused by free radicals as a result of diabetes (21,22). Fe2+ converts into Fe^{3+} in vivo conditions and during Fenton reaction system. Regenerating Fe^{2+} from Fe^{3+} is occurred by ascorbate which contemporary the lipid peroxidation is performed as well. In this case, treatment by alpha LA reduced the peroxidation process and it can prevent reducing the level of antioxidants (21,22). It has reported that alpha LA may reduce the lipid peroxidation by metals chelating effect (23). Glutathione is the most

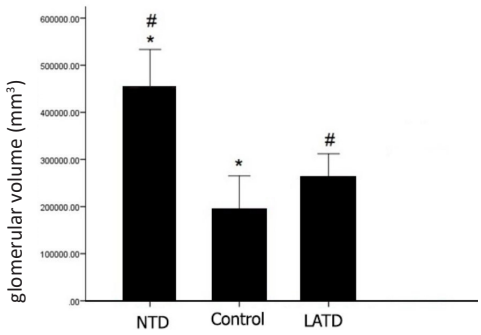

Figure 2. The effect of alpha lipoic acid on glomerular volume in alloxan induced diabetic rats.
*P< 0.050 as comparison between control and NTD (diabetic group), # P< 0.050 as comparison between NTD and LATD (alpha lipoic acid treatment group).

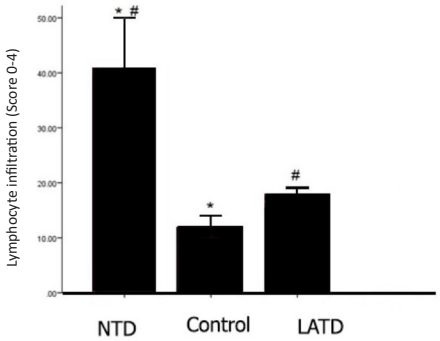

Figure 3. The effect of alpha lipoic acid on lymphocyte infiltration in alloxan induced diabetic rats. Lymphocyte infiltration was determined by light microscopy on formalin-fixed cortical sections. Results are expressed as means ± SEM of 1000 field per group.
*P< 0.050 as comparison between control and NTD (diabetic group), # P< 0.050 as comparison between NTD and LATD (alpha lipoic acid treatment group).

abundant enzymatic antioxidant in mammalian cells. Increased oxidative stress caused by frequent variations in glutathione density which makes beta and pathogenesis cell dysfunction of long-term diabetes complications and abnormalities in the protection of NADPH and consequently makes it possible to active polyol pathway (21-23). This performance of glutathione and other antioxidants perform an important function in limiting free radical reactions that it causes a severe lipid peroxidation if it is not performed (21-23). Alpha LA performs an important function in improving the glutathione condition. Alpha LA can increase GSH form it GSSG. It is said that existing glutathione is essential to prove the alpha LA antioxidant effects (23-25). It should be noted that alpha LA may have a good effect on glutathione cycle and maintain cellular glutathione by impacting on glutathione reductase (25). DN is known as a common manifestation in advanced stages of diabetes (26). Morphological lesions are mainly observed in glomeruli including thickening of glomerular basement membrane (GBM) and mesangial expansion (26). Several mechanisms such as formation of advanced glycation end products (AGEs), excessive formation of ROS, activation of protein kinase C (PKC) and renin-angiotensin system (RAS), are postulated to contribute to the development and progression of DN (26,27). Our findings of increased mean glomerular volume and lymphocyte infiltration clearly indicate progressive kidney dysfunction. Glomerular size is considered as an indicator to determine the rate of decline in kidney function in the diabetic patient (26). Endothelial dysfunction is associated with development and progression of renal pathology in patients with type 1 diabetes (26,27). Our finding of elevated activity of MPO enzyme in serum, liver and kidney showed increased risk of cardiovascular and renal complications. MPO plays as a potent oxidant to kill microbial agents. However it causes damage at inflammatory site which our histopathological findings confirmed it. LA treatment was highly effective in attenuating renal lesions in treated rats. Likewise, MPO activity was markedly reduced. LA was found even more effective than vitamin E and vitamin C to prevention early glomerular injury induced by diabetes (28). Since hydrogen peroxide acts as a substrate for MPO, it is believed that enhanced production of hydrogen peroxide in diabetic patients is responsible for MPO improved activity (29). These findings indicate that LA strengthens antioxidant defenses and protect the kidney from damages induced by excessive ROS production.

Our finding showed no difference in GPx and Cu–ZnSOD gene expression in kidney tissue of diabetic rats. There are various reports about effect of oxidative stress on antioxidant gene expression. For example there are reports of increased (30), decreased (31) or even unchanged (32) expression of GPx gene in diabetic rats. These variations may be explained by differences in the experimental conditions, such as time since onset of diabetes and age of the experimental animals. Arambašić et al (33) reported marked reduction in the activities of MnSOD, Cu-ZnSOD and CAT in kidneys of diabetic rats. Likewise, our study revealed a decrease in CAT gene expression in the kidney of diabetic rats. Oxidative stress induced by diabetes is believed to cause oxidizing of transcriptional factors required in transcription process, shortening the half-life of mRNA and structural alterations of mRNA (34). Alpha-LA treatment effectively restored expression of GPx, CAT and Cu-ZnSOD genes in LA-treated group.

6. Conclusions

Besides its beneficial role as a potent antioxidant, LA serves as a coenzyme for different mitochondrial enzymes. Therefore, desired effects of LA are not attributed only to antioxidant property. The combination of antioxidant and metabolic roles are likely to be involved in protective effects of LA. In conclusion, we demonstrated that LA protects against diabetic induced oxidative stress by restoring the biochemical alterations, amelioration of renal lesions and modulation of antioxidant gene expression levels, and hence could be considered as a suitable candidate to prevent diabetes complication.

Acknowledgments

The authors wish to thank Deputy of Research and Razi Herbal Research Center of Lorestan Medical University, Lorestan, Iran.

Authors' contribution

HA designed the project. PJ collected the data. HA, PJ and HA analyzed the data. HA and PJ wrote the manuscript. HA and PJ revised English version. HA edited the final draft. All authors read and signed the final manuscript.

Conflicts of interest

The authors declare no conflict of interest

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

This research was supported by Lorestan University of

Medical Sciences (Grant# 21/91).

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27:1047- 1053. doi: 10.2337/diacare.27.5.1047
2. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. Sultan Qaboos Univ Med J. 2012;12(1):5-18.
3. Davì G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. Antioxid Redox Signal. 2005;7(1-2):256-68. doi: 10.1089/ars.2005.7.256
4. Domínguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative Stress at Onset and in Early Stages of Type 1 Diabetes in Children and Adolescents. Diabetes Care. 1998;21(10):1736-42.
5. Pourkhodadad S, Alirezaei M, Moghaddasi M, Ahmadvand H, Karami M, Delfan B, Khanipour Z. Neuroprotective effects of oleuropein against cognitive dysfunction induced by colchicine in hippocampal CA1 area in rats. J Physiol Sci. 2016;66(5):397-405. doi: 10.1007/s12576-016-0437-4.
6. Wolff SP. Diabetes mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull. 1993;49(3):642-52.
7. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med. 2006;40(2):183-92. doi:10.1016/j.freeradbiomed.2005.06.018
8. Wiersma JJ, Meuwese MC, van Miert JN, Kastelein A, Tijssen JG, Piek JJ, Trip MD. Diabetes mellitus type 2 is associated with higher levels of myeloperoxidase. Med Sci Monit. 2008;14(8):CR406-10.
9. Yi X, Nickeleit V, James LR, Maeda N. α-Lipoic Acid Protects Diabetic Apolipoprotien E-deficient Mice from Nephropathy. J Diabetes Complications**.** 2011;25(3):193-201. doi:10.1016/j.jdiacomp.2010.07.004.
10. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review J Biochem Mol Toxicol. 2003;17(1):24-38. doi: 10.1002/jbt.10058.
11. Winiarska K, Malinska D, Szymanski K, Dudziak M, Bryla J. Lipoic acid ameliorates oxidative stress and renal injury in alloxan diabetic rabbits. Biochimie. 2008;90(3):450-9. doi: 10.1016/j.biochi.2007.11.010
12. Obrosova IG, Fathallah L, Liu E, Nourooz-Zadeh J. Early Oxidative Stress in the Diabetic Kidney: Effect of DL-alpha-lipoic Acid. Free Radic Biol Med. 2003;34(2):186-95.
13. Tavafi M, Ahmadvand H, Khalatbari A, Tamjidipoor A. Rosmarinic Acid Ameliorates Diabetic Nephropathy in Uninephrectomized Diabetic Rats. Iran J Basic Med Sci. 2011;14(3): 275-83.
14. Ahmadvand H, Tavafi M, Khosrowbeygi A. Amelioration of altered antioxidant enzymes activity and glomerulosclerosis by coenzyme Q10 in alloxan-induced diabetic rats. J Diabetes Complications. 2012;26(6):476-82. doi: 10.1016/j.jdiacomp.
15. Khalatbary AR, Ahmadvand H. Effect of oleuropein on tissue myeloperoxidase activity in experimental spinalcord trauma. Iran Biomed J. Iran Biomed J. 2011;15(4):164-7.
16. Ani M, Moshtaghie AA, Ahmadvand H. Comparative effects of copper, iron, vanadium and titanium on low density lipoprotein oxidation in vitro. Iran Biomed J. 2007;11(2):113-118.
17. Ahmadvand H, Noori A, Ghasemi Dehnoo M, Bagheri S, CheraghiRA. Hypoglycemic, hypolipidemic and antiatherogenic effects of oleuropein in alloxan-induced Type 1 diabetic rats. Asian Pac J Trop Dis. 2014;4(1):S421-S425. doi:10.1016/S2222-1808(14)60481-3.
18. Dal S, Sigrist S. The Protective Effect of Antioxidants Consumption on Diabetes and Vascular Complications. Diseases (Basel, Switzerland) 2016;4(3):24. doi: 10.3390/diseases4030024.
19. Ndahinana J, Dorchy H, Vertongen F. Erythrocyte and plasma antioxidant activity in diabetes mellitus type 1. Presse Med. 1996;25:188–92.
20. Kono Y, Fridovich I. Superoxide radical inhibits catalase. J Biol Chem. 1982;257(10):5751-4.
21. Jones W, Li X, Qu ZC, Perriott L, Whitesell RR, May JM. Uptake, recycling, and antioxidant actions of alpha-lipoic acid in endothelial cells. Free Radic Biol Med. 2002; 33(1):83-93.
22. Nickander KK, McPhee BR, Low PA, Tritschler H. Alpha-lipoic acid: antioxidant potency against lipid peroxidation of neural tissues in vitro and implications for diabetic neuropathy. Free Radic Biol Med. 1996; 21(5):631-9.
23. Matsugo S, Yan LJ, Han D, Trischler HJ, Packer L. Elucidation of antioxidant activity of alpha-lipoic acid toward hydroxyl radical. Biochem Biophys Res Commun. 1995; 208(1):161-7. doi: 10.1006/bbrc.1995.1318
24. Zatalia SR, Sanusi H. The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. Acta Med Indones. 2013;45(2):141-7.
25. Pick U, Haramaki N, Constantinescu A, Handelman GJ, Tritschler HJ, Packer L. Glutathione reductase and lipoamide dehydrogenase have opposite stereospecificities for α-lipoic acid enantiomers. Biochem Biophys Res Commun. 1995;206(2):724-30. doi: 10.1006/bbrc.1995.1102.
26. Tavafi M, Ahmadvand H, Tamjidipoor A, Delfan B, Khalatbari AR. *Satureja khozestanica* essential oil ameliorates progression of diabetic nephropathy in uninephrectomized diabetic rats. Tissue Cell. 2011;43:45-51. doi: 10.1016/j.tice.2010.11.004.
27. Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des. 2005;11(18):2279-99.
28. Melhem MF, Craven PA, Derubertis FR. Effects of dietary supplementation of lipoic acid on early glomerular injury in diabetes mellitus. J Am Soc Nephrol. 2001;12(1):124-33.
29. Yi X, Nickeleit V, James LR, Maeda N. α-Lipoic Acid Protects Diabetic Apolipoprotien E-deficient Mice from Nephropathy. J Diabetes Complications. 2011;25(3):193-

201. doi: 10.1016/j.jdiacomp.2010.07.004.
30. Matsunami T, Sato Y, Sato T, Ariga S, Shimomura T, Yukawa M. Oxidative stress and gene expression of antioxidant enzymes in the streptozotocin-induced diabetic rats under hyperbaric oxygen exposure. Int J Clin Exp Pathol. 2009;3:177-88.
31. Manna P, Das J, Ghosh J, Sil PC. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: Prophylactic role of arjunolic acid. Free Radic Biol Med. 2010;48(11):1465-84. doi: 10.1016/j.freeradbiomed.2010.02.025.
32. Sadi G, Güray T. Gene expressions of Mn-SOD and GPx-1 in streptozotocin-induced diabetes: effect of antioxidants. Mol Cell Biochem. 2009; 327(1-2):127-34. doi: 10.1007/s11010-009-0050-4.
33. Arambašić J, Mihailović M, Uskoković A, Dinić S, Grdović N, Marković J, et al. Alpha-lipoic acid upregulates antioxidant enzyme gene expression and enzymatic activity in diabetic rat kidneys through an O-GlcNAc-dependent mechanism. Eur J Nutr. 2013; 52(5):1461-73. doi: 10.1007/s00394-012-0452-z.
34. Sadi G, Yilmaz O, Güray T. Effect of vitamin C and lipoic acid on streptozotocin-induced diabetes gene expression: mRNA and protein expressions of Cu–Zn SOD and catalase. Mol Cell Biochem. 2009; 327(1-2):127-34. doi: 10.1007/s11010-009-0050-4.