Effect of Vitamin E on Blood Glucose, Insulin, Lipid Peroxides, and Antioxidant System of Streptozotocin-Induced Diabetes in Rats

Amel L El-Sawaf*, Hassan M. Fayed*

Abstract: Background: There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia, causes oxidative stress which further exacerbates the development and progression of diabetes and its complications. There are multiple sources of oxidative stress in diabetes including non-enzymatic, enzymatic, and mitochondrial pathways. Vitamin E is a fat-soluble vitamin that prevents lipid peroxidation. **Objective:** the present study was carried out to test the effect of vitamin E on blood glucose, insulin, and lipid peroxides in blood and liver tissue of rats in relation to oxidative damage associated with diabetes induced by streptozotocin (STZ). **Methods:** 24 male albino rats were randomly assigned to control (group I), streptozotocin (STZ)-induced diabetic rats (group II), the third group (vitamin E group) were STZ-induced diabetic rats fed 400 mg of vitamin E/kg diet. After 4 weeks of the induction of diabetes, rats were sacrificed and the following determinations were done on the blood, serum or plasma. Blood glucose, serum insulin, lipid peroxide concentration in plasma as malonyldialdehyde (MDA) level in nmol/g protein, the amount of thiobarbituricp acid reactive materials in plasma (TBARM), serum antioxidant capacity (assayed by measuring the total peroxy radical trapping capacity (TRAP) of serum, and serum superoxide dismutase, enzyme activity (SOD). In the liver, the following parameters were determined: liver MDA, SOD and Glutathione peroxidase (GSH-Px) enzyme activaties, and Glutathione (GSH) concentration. **Results:** Hyperglycemia, hypoinsulinemia were regarded in group II which were ameliorated by vitamin E administration. Oxidant stress was found in diabetic rats group II manifested by increase concentration of MDA-plasma and liver, increase TBARM concentration, and TRAP-plasma and serum respectively. Also increased serum SOD, liver SOD, and GSH-Px enzyme activities in these diabetic rats. Administration of vitamin E in the diet decreased the oxidant stress parameters (MDA, TBARM, and TRAP), increased the antioxidant defense parameter (increased GSH concentration in liver), and decreased the oxidant stress as manifested by the decrease in serum SOD enzyme activity; liver SOD; and GSH-Px enzyme activities. **Conclusion:** Vitamin E was found to be excellent for strengthening the antioxidant defense system in STZ-diabetic rats and it may therefore have a therapeutic role in combating the damaging effect of ROS in diabetes and preventing its complications.

INTRODUCTION

Elevated glucose levels are associated with increased production of reactive oxygen species (ROS) mainly through the glycation reaction which occurs in various tissues.(1) In addition, superoxide $\left(^{-}O_2^{\bullet}\right)$ is generated by the process of glucose auto-oxidation that is associated with formation of glycated proteins in the plasma of diabetic patients.(2) The interaction of advanced glycation end products with corresponding cell surface receptors stimulates ROS production and decreases intracellular glutathione levels.(2) The increase in ROS production contributes to the development of diabetic

*Departments of Physiology and Biochemistry Medical Research Institute, Alexandria University

complications such as atherosclerosis and other vascular complications. Therefore, treatment with antioxidants may have a place in ameliorating diabetic complications. It is argued now, whether superoxide antioxidant defense and cellular redox status should be regarded as central players in the diabetic and metabolic syndrome.[2] Antioxidant therapies including the gene transfer of antioxidant enzymes are therefore potentially valuable in diabetic treatment.[3]

The present study was designed to investigate the role of vitamin E as an antioxidant supplemented to rat's diet in the amelioration of lipid peroxidation process, in both the blood and liver of streptozotocin (STZ)-induced diabetic rats and its role in strengthening the antioxidant system of them.

MATERIAL AND METHODS

Twenty-four male albino rats (200-250 g.) were used in the present study. All rats were fed rat chow and water. They were divided randomly into 3 groups.

Group I (n = 8) is the control group, which was injected with sterilized buffer.

Group II (n = 8) was made diabetic by single intraperitoneal injection of streptozotocin (STZ) 40 mg/kg[4] body wt. Diabetes was verified by hyperglycemia which developed 3 days after STZ injection.

Group III was diabetic-induced rats like group II but received α-tocopherol (vitamin E) with diet (400 mg/kg rat chow).[5]

Rats were sacrificed by decapitation 4 weeks later. Blood was collected. Part of the blood was left to clot and centrifuged at 3000 r.p.m for 30 minutes to separate the serum. The remaining blood was mixed with EDTA to separate plasma.

The liver was separated, rinsed in ice cold phosphate buffer saline (PBS pH 7.4), blotted by filter paper and weighed. A 20% w/v homogenate was prepared in a 0.25 mol/L sucrose solution using a Potter-Elvehjem glass, Teflon homogenizer driven by an electric motor at 500 r.p.m.

Serum, plasma, and liver samples were kept at -70°C till analysis. Supernatant fractions from liver homogenates were prepared by centrifugation at 10,000 g. for 10 min at 4°C.[6] Protein concentrations in liver homogenates supernatants were estimated by the method of Lowry *et al*.[7]

Blood glucose was determined by glucose oxidase method[8]. Serum insulin assay was determined by radioimmunoassay[9] technique by a kit purchased from Incstar Diagnostic Cooperation.

Assay of lipid peroxidation products in plasma or serum and liver

The level of oxidative stress was estimated by the following methods:

1- Measuring the amount of lipid peroxides in plasma and liver. The amount of thiobarbituric acid reactive materials (TBARM) in plasma was measured by the colorimetric method of Bird and Draper.[10]

2- Malonyldialdehyde (MDA) levels in plasma and liver supernatant fraction was measured by the method of Ohkawa *et al.,*[11] and expressed as nmol MDA/g protein.

3- Serum antioxidant capacity was assayed by measuring the total peroxy radical trapping capacity (TRAP) of serum. The analysis was based on the ability of serum to trap free peroxy radicals generated from azobis[12] (2-amidinopropane/hydrochloride).

The antioxidant system activity was determined by:

1- Serum superoxide dismutase (SOD) enzymatic activity was assayed according to the method of Paolotti *et al*.[13]

2- Evaluation of liver free radical scavenging capacity was carried out through measuring:

   a- Glutathione content[14] (GSH).

   b- Glutathione peroxidase enzyme activity[15] (GSH-Px).

c- SOD activity of liver.[16]

STATISTICAL ANALYSIS

The results of the present study was statistically analyzed using Statistical Package for Social Sciences (SPSS)[17] for Windows version 9.0 software.

RESULTS

No difference in food intake or body weight was observed among the 3 groups (group I: control, group II: STZ-induced diabetics, and group III: Diabetic + vitamin E supplementation group).

STZ in the given dose induced diabetes in groups II & III animals as manifested by hyperglycemia and hypoinsulinemia as compared to group I ($p < 0.001$), table 1.

Diabetic group showed increased serum LP, TBAR values, total peroxy-radical-trapping capacity, and LP value of the liver compared to controls ($p<0.001$) indicating increased oxidant stress, table 2.

Diabetic group showed increased serum and liver SOD and GSH-Px enzyme activities, ($p<0.001$) and decreased liver GSH concentration, table 3. Administration of vitamin E with diet (400 mg/kg diet) (group III) resulted in highly significant decrease of serum glucose ($p<0.001$) as compared to group II but is still significantly higher than controls also serum insulin of group III increased significantly when compared to diabetics but still below the mean value of the controls.

Vitamin E administration to diabetic rats group III ameliorated the oxidant stress as evidence by decreased values of oxidant markers (serum LP, TBARM, TRAP, and liver LP) compared to diabetic group but still significantly higher for TBARM, TRAB, and liver LP ($p<0.001$) when compared to control values. However, LP of serum did not change significantly ($p>0.05$) as compared to control value, table 2.

Antioxidant enzymes activities (serum SOD, liver SOD & liver GSH-Px) of group III showed significant decrease as compared to

diabetic group II while it was still significantly higher than control values. Liver GSH concentration increased significantly in group III compared to diabetic group II. It was significantly higher than control value (group I), table 3. The above findings denote decreased oxidant stress after supplementation of vitamin E to diet.

DISCUSSION

There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia causes oxidative stress,[1] which further exacerbates the development and progression of diabetes and its complications. Overproduction and/or insufficient removal of these free radicals results in vascular dysfunction, damage to cellular proteins, membrane lipids, and nucleic acids.[2]

There are multiple sources of oxidative stress in diabetes including non-enzymatic, enzymatic, and mitochondrial pathways. Non-enzymatic sources of oxidative stress in diabetes are the oxidative process of glucose. Hyperglycemia recorded in the present study of STZ diabetic rats can directly cause increased ROS generation.[1] Glucose can undergo autoxidation and generate •OH radicals.[18] In addition, glucose reacts with proteins in a non-enzymatic manner leading to the development of advanced glycation end products (AGE).[1,18] ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of superoxides $\left({}^{-}O_2^{\bullet} \right)$.[18]

Enzymatic sources of augmented generation of reactive species in diabetes include nitric oxide synthase (NOS) enzyme, NAD (P) H oxidase and xanthine oxidase.[19-21] If NOS lacks its substrate L-arginine or one of its co-factors, NOS may produce $\left({}^{-}O_2^{\bullet} \right)$ instead of NO.[19,20] NAD (P)H oxidase is a membrane associated enzyme which is a major source of $\left({}^{-}O_2^{\bullet} \right)$ production.[19,20]

There is enhanced production of $\left(^{-}O_2^{\bullet}\right)$ in diabetes predominantly produced by NAD (P) H oxidase.[19]

The mitochondrial respiratory chain is another source of non-enzymatic generation of reactive species. A recent study demonstrated that hyperclycemia-induced generation of $\left(^{-}O_2^{\bullet}\right)$ at the mitochondrial level was the initial trigger of the vicious cycle of oxidative stress in diabetes.[22,23]

The present results (Table 2) show highly significant increase (p<0.001) of MDA as a measure of lipid peroxidation in the plasma of STZ diabetic rats as a result of oxidative stress. This finding is in accordance with multiple previous results.[24-29]

Plasma TBARM and serum TRAP levels as indirect measurement of free radical production have been also shown to be constantly elevated in diabetes as recorded in previous studies[26-28] and in the present work, (Table 2)

The effect of antioxidant system of the body on the oxidation stress can be measured through certain observable biomarkers. These markers include the enzyme activities of SOD, GSH-Px, and GSH-reductase as well as GSH concentration. The enzymes studied (SOD in serum and liver, GSH-Px in liver) were found to be highly significantly elevated (p<0.001) which is in accordance with previous studies.[26,27,30] GSH which acts as a direct scavenger as well as a co-substrate for GSH-Px was recorded to be decreased in the present study in the liver as recorded previously.[26,27,30]

Contrary to the present results, GSH-Px expression in experimental activity were shown to be decreased in experimental models of diabetes.[21,28,31-33] However, Ulusu *et al.,* (2003)[27] found that GSH-Px was increased by STZ diabetic rats in brain, heart, and kidney. Hunkar *et al*., (2002)[25] recorded an increased activity of the enzyme in the aorta, heart, and liver. The same enzyme activity was found to be increased in the brain, heart, and kidney[20], and RBCs.[16] However,

Waziroglu and Cay (2001)[24] found no significant change of GSH-Px activity in RBC, liver, and muscle of STZ diabetic rats compared to their controls.

It may be said that the most prominent defense enzymes against the oxidant stress are SOD, catalase enzymes which together with GSH-Px constitute the major intracellular antioxidant protective system by removing the superoxide anion and H_2O_2.[1] The increase of enzyme activity of SOD and GSH-Px in the liver in the present work denotes marked oxidative stress in the STZ-diabetic animals.

Although animals have their own antioxidant defense systems, the defense can be extremely strengthened. Vitamin E is a well known dietary antioxidant. Vitamin E is lipophilic and inhibits lipid peroxidation scavenging lipid peroxyl radicals to yield lipid hydroperoxides and the tocopheroxyl radical.[1] Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back to the phenol by ascorbate and NAD (P)H-dependent reductase enzymes.[1]

In the present study, it is evident that antioxidant treatment with vitamin E can improve the glycemic control with preservation of pancreatic β-cell function of STZ diabetic rats by increasing the level of insulin (Table 1). The antioxidant treatment probably exerts its effects in association with the presence of hyperglycemia, i.e., by protecting β-cells from the toxic effects of ROS produced under hyperglycemic condition.

Kaneto *et al*., (1999)[1] found that the antioxidant treatment increased the β-cell mass and preserved its insulin content, using immunostaining technique for pancreatic sections of STZ rats treated with antioxidants. Recently Al-Shamsi *et al*., (2006)[34] found that vitamin E significantly increased plasma insulin level in normal rats but failed to increase the plasma insulin level in diabetic rats. In the present study, vitamin E indeed reduced blood glucose level and increased insulin level (Table 1). Thus vitamin E may play a role in glucose

metabolism and thus be a useful adjuvant therapy in type I diabetes.[35] In the plasma and liver, it decreased lipid peroxides (MDA) and SOD enzyme activity, GSH-Px enzyme activity in liver, and increased GSH concentration in liver (Tables 2 & 3). Similar results were obtained by several authors,[27,33,36-42] who found that vitamin E was effective in reducing several indices of oxidative stress including lipid peroxides. Also the present study showed decrease of other oxidant stress markers, i.e., plasma TBARM and serum TRAP (Table 2).

Maritim *et al.,* (2003)[43] using another antioxidant (α-lipoic acid) recorded similar results in STZ-induced diabetes.

Concerning the mechanism of action of vitamin E, Garg and Bansal (2000)[44] suggest that vitamins C and E reduce lipid peroxidation by quenching free radicals. Hong *et al.,* (2004)[5] suggest that vitamin E reduces the generation of ROS and damages the oxidative substances.

Kaneto *et al*., (1999)[1] suggest that as antioxidants administration reduced blood glucose level it must have reduced glycose hyperglycemic toxicity by preventing the vicious circle of generation of ROS and further damage of β-cells and hyperglycemia, and moving the cycle in the opposite direction. Also, they suggest that there is a possibility that the antioxidant treatment could have exerted an influence on target tissues other than the β-cells.[1]

The present results therefore clearly demonstrates that vitamin E supplementation augments the antioxidant defense mechanism in diabetes, and provides evidence that vitamin E may have a therapeutic role in combating free radical mediated disease

Table (1): Serum glucose and insulin levels in control, STZ diabetic rats, and diabetic rats supplemented with vitamin E

	Blood Glucose (mg/dl)	Serum Insulin (Pmol/L)
Control (n = 8) $\overline{X}$ ±S.E	88.71±1.28	330±7.03
Diabetic (n = 8) $\overline{X}$ ±S.E	276.3±7.54*	82.5±1.7*
Diabetic + vitamin E (n = 8) $\overline{X}$ ±S.E	109.3±0.73*a,b	142.37±3.48*a,b

* $p_1 < 0.001$ Diabetic Vs control.
a $p_2 < 0.001$ Diabetic + vitamin E Vs control.
b $p_3 < 0.001$ Diabetic + vitamin E Vs Diabetic.

Table (2): Serum and liver lipid peroxidation product's concentrations and capacity in control, STZ-induced diabetic rats, and diabetic + vitamin E supplemented rats

	Plasma LP (nmol MDA/g. protein)	Plasma TBARM (µmol/L)	Serum TRAP (µmol/L)	Liver MDA (nmol MDA/g. protein)
Control (n = 8) $\overline{X}$ ±S.E	0.6±0.02	1.35±0.01	6.45±1.4	2.03±0.01
Diabetic (n = 8) $\overline{X}$ ±S.E	1.42±0.03*	2.54±0.01*	9.69±4.42*	3.92±0.01*
Diabetic + vitamin E (n = 8) $\overline{X}$ ±S.E	N.S 0.6±0.01	1.76±0.01*a,b	6.95±1.64*a,b	2.62±0.0*a,b

* $p_1 < 0.001$ Diabetic Vs control.
a $p_2 < 0.001$ Diabetic + vitamin E Vs control.
b $p_3 < 0.001$ Diabetic + vitamin E Vs Diabetic.
N.S > 0.05 non-signifiant Diabetic + vitamin E Vs control.

Table (3): Effect of STZ-induced diabetes, vitamin E supplementation to STZ diabetic rats on antioxidant parameters in serum and liver

	Serum SOD (U/mg protein)	Liver GSH-Px nmol NADPH oxidized/min/ mg protein	Liver SOD (U/mg. protein)	Liver GSH µmol/mg. protein
Control (n = 8) $\overline{X}$ ±S.E	5.8±0.01	342±0.75	5.62±0.01	5.83±0.01
Diabetic (n = 8) $\overline{X}$ ±S.E	9.33±0.05*	489.9±0.75*	7.3±0.12*	2.83±0.01*
Diabetic + vitamin E (n = 8) $\overline{X}$ ±S.E	7.09 ± 0.02*a,b	369±0.69*a,b	5.95±0.01*a,b	4.92±0.00*a,b

*p_1 < 0.001 Diabetic Vs control.
a p_2 < 0.001 Diabetic + vitamin E Vs control.
b p_3 < 0.001 Diabetic + vitamin E Vs Diabetic.

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