Journal of Pharmacy and Pharmacology 5 (2017) 708-716
doi: 10.17265/2328-2150/2017.010.002

Protective Effects of Vitamin E on Diabetes-induced Oxidative Stress Status and Homocysteine in the Rat Heart

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Abstract: Objective: We aimed to investigate protective effects of vit E on oxidative stress status and homocysteine (Hcy) in cardiac tissue of diabetic rats. Methods: Sixteen Wistar male rats were treated with STZ (streptozotocin) (60 mg/kg) to induce diabetes. Diabetic rats were divided into two groups: NTD (non-treated diabetic) and V_ETD (vit E-treated diabetic) rats. The V_ETD group received 300 mg/kg vit E with daily feeding. Eight normal rats of the same age were used as the control group. After 6 weeks, the rats were anesthetized, their cardiac tissue was removed, and homogenated supernatant was separated. Samples were assayed for TAC (total antioxidant capacity), LPO (lipid peroxidation), nitrite (NO_2^-), nitrate (NO_3^-), and Hcy. Key Findings: The contents of LPO, NO_3^- and Hcy in NTD compared to control group indicate a significant increase, but the levels of these parameters decreased in V_ETD ($p < 0.05$). There was a significant decrease in the amount of TAC in the NTD group but in V_ETD group, that significantly increased ($p < 0.05$). The amount of NO_2^- in NTD and V_ETD groups, compared to the control group, did not show any significant changes ($p > 0.05$). Conclusions: Significant decrease of oxidative stress and Hcy in the cardiac tissue caused by vit E supplementation strongly indicated that this radical scavenger may promote a protective effect on diabetic cardiomyopathy through the attenuation of oxidative stress and increase antioxidant defense mechanism.

Key words: Vitamin E, oxidative stress, homocysteine, diabetic rats.

1. Introduction

Diabetes mellitus has been widely recognized to be a fundamental and leading cause of major health issues, especially of all the cardiovascular diseases. The majority of diabetes-related deaths arise from cardiovascular complications such as myocardial infarction, stroke, and peripheral vascular disease [1-3]. Some evidence shows that oxidative stress is associated with the pathogenesis of diabetes, hypertension, cardiovascular diseases, heart failure, and mitochondrial disease [4, 5].

Oxidative stress may play an important role in the development of vascular complications in diabetes [6]. It is increased in diabetic patients and this may lead to the production of ROS (reactive oxygen species) and a rapid decrease in defensive antioxidants. The generation of ROS in diabetes occurs via several mechanisms and is initiated not only by glucose, but also by other substances that are found at elevated levels in diabetic patients [2, 7]. This causes an increase in the level of plasma lipid peroxides and leads to diseases such as atherosclerosis [3]. In diabetic patients, lipid peroxidation initiates the development of a chain reaction, which causes cell damage.

On the other hand, Hcy (homocysteine) is known to participate in the development of atherosclerosis and vascular damage and it has been suggested to contribute to the atherosclerotic process of diabetes [8, 9] . Homocysteine promotes oxidant injury to vascular

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cells through the auto-oxidation, formation of Hcy mixed disulfides, interaction of Hcy thiolactones and protein homocysteinylation [4, 10]. Several effects produced by Hcy on vascular cells have been described, such as diminished NO (nitric oxide) released from endothelial cells [11], increased ROS [12], potentiation of low-density lipoproteins oxidation [13]. Also, it was shown that Hcy decreases NO content enhancing ROS generation [14].

In addition, certain antioxidants, such as vitamin E (vit E), a fat-soluble vitamin and potent chain-breaking antioxidant helps to prevent damage to the lipids by the free oxygen radicals mediated tissue injuries in diabetes [15] and it acts as the first line of defense against lipid peroxidation, protecting the cell membranes from free radical attack [16]. A study by Halliwell (2002) demonstrated that vit E contributes to a delay of insulin resistance in diabetic rats [17]. Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins. A number of studies have been carried out to determine the protective effect of vit E in different biological models of injury [18]. Thus, our study was undertaken to determine whether the heart is subject to oxidative damage during diabetes and also to determine the possible protective effect of vit E against diabetes induced alterations of heart injuries as well as to evaluate the accompanying changes in Hcy levels in order to understand its role in the heart of diabetic rats.

2. Material and Methods

2.1 Animal Treatment

Sixteen male white Wistar rats, weighing 220-240 g, were made diabetic by intraperitoneal injection of streptozotocin [(STZ): 60 mg/kg body weight in 0.05 M citrate buffer, pH = 4.5]. Serum glucose was determined by glucose oxidase method using kit (Biosystem Co., Spain) on blood samples obtained from tail veins 48 h after injection of STZ. Rats with blood glucose higher than 300 mg/dL were included in the study as diabetics. Then, these rats were divided into two groups: NTD (non-treated diabetic) and vit E-treated diabetic V_ETD groups. Rats in the V_ETD group received 300 mg/day vit E (Merck Co., Germany) in tap water in addition to their daily regular diet. Age- and weight-matched normal rats (n = 8) were injected intra- peritoneal with an equivalent amount of 0.05 M citrate buffer at pH = 4.5. After 6 weeks, all rats were anesthetized with 10% chloral hydrate (5 mL/kg body weight). Then, the abdominal cavity was opened and the whole heart was harvested. The heart was segmented and each segment was flushed with chilled KCl (115 g/L) solution. A cardiac tissue homogenate (10% W/V) was prepared in 50 mM phosphate buffer (pH = 7.2) and then centrifuged at 10,000 × g for 10 min at 4 °C in a refrigerated centrifuge (Hermel Co., Germany). The obtained supernatant was used for all the assays.

The study protocol was approved by Ethical Committee of the veterinary faculty of Islamic Azad University and Medical Ethics Committee of Urmia University of Medical Sciences, Iran, adopted from current version of declaration of Helsinki.

2.2 Measurements of Oxidative Stress Markers

Lipid hydroperoxides were measured using reductive reaction involving ferrous ion using kit (Cayman, USA). Briefly, hydroperoxides unstably increase and immediately react with the ferrous ion and produce ferric ion. Ferric ion is detected by thiocyanate ion, which is a chromogen. TAC (total antioxidant capacity) measurement was performed using calorimetric method (Randox Laboratories Co., UK). In this method, ABTS (2, 2'-Azinobis, 3-ethylbenzothiazoline-6-sulfonic acid di-ammonium salt) is incubated together with met-myoglobulin (as a peroxidase) and hydrogen peroxide and produces the cationic ABTS radical. This radical has a rather stable greenish blue color which can be of measured at a wavelength of 600 nm. Proportional to their concentrations, antioxidants in the sample decrease the intensity of the color. Nitrite and nitrate assays were

performed using (Cayman Co., USA) kit.

2.3 Measurement of Homocysteine

The tissue Hcy levels were measured enzymatically (Diazyme, USA) using an auto-analyzer system BT3000 (Bioelectronica, Italy). Briefly, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, SAM (S-adenosyl methionine), catalyzed by an Hcy S-methyltransferase. The co-substrate conversion product is amplified by coupled enzymatic cycling reactions. The total Hcy level in the sample is indirectly proportional to the amount of NADH conversion to NAD^+.

2.4 Statistical Analysis

A one-way analysis of variance (ANOVA) test was used to compare the values among groups. The data were expressed as mean ± SD, and $p < 0.05$ was considered statistically significant.

3. Results

Glucose, HbA1C and total protein values of control group, NTD and vitamin E-treated diabetic group (V_ETD) were shown in Table 1. Blood glucose is evaluated in the beginning of experiment, after 48 hours of STZ injection and at the end of the experiment. Body gain is decreased significantly in NTD group compared to the VETD group ($p = 0.002$). However, there is not a significant change in VETD group compared to the control ($p = 0.09$). Glucose values significantly increased in 48 hours after STZ injection ($p = 0.005$). However, in the end of experiments, it decreased significantly in V_ETD group than NTD and 48 hours after STZ injection ($p < 0.05$). HbA_1C value is increased significantly in NTD group ($P = 0.005$) but it was the same as normal in V_ETD group (Table 1).

Compared to the control group, a significant increase was shown in NO_3^- content in the NTD group (12.75 ± 1.9 μmol/L vs. 35.85 ± 3.4 μmol/L; $p < 0.05$). However, there was a significant decrease in V_ETD rats (17.65 ± 2.1 as compared to NTD group, $p < 0.05$, Fig. 1). As shown in Fig. 2, NO_2^- content in NTD and V_ETD animals did not change compared to the control group (4.65 ± 1.0, 4.85 ± 1.0 and 4.55 ± 0.9 μmol/L, respectively, $p > 0.05$).

A significant decrease in NO_2^-/NO_3^- ratio was seen in the control group compared to the V_ETD and NTD groups (0.38 ± 0.10, 0.127 ± 0.02 and 0.277 ± 0.1, respectively, $p < 0.05$.

A significant increase in NO_2^-/ NO_3^- ratio was also seen between V_ETD group and NTD group, $p < 0.05$; but there was no significant difference in NO_2^-/NO_3^- ratio between the V_ETD and the control group; $p > 0.05$, Fig. 2.

Our research results indicate a significant decrease in TAC content in the NTD group compared to the control group (3.3 ± 0.8 and 0.7 ± 0.05 μmol/L, respectively); $p < 0.05$. After treating with vit E, there was a significant increase in TAC content (1.4 ± 0.4 μmol/L) compared to diabetic rats; $p < 0.05$. However, a significant decrease was found compared to the control group; $p < 0.05$, Fig. 3.

A significant increase was seen in LPO content in the NTD group (5.56 ± 0.5 μmol/L) compared to controls (1.84 ± 0.3 μmol/L); $p < 0.05$. After treating with vit E, there was a significant decrease in LPO content compared to diabetic rat (2.89 ± 0.7 μmol/L); $p < 0.05$, but a significant increase was shown compared to the control group; $p < 0.05$, Fig. 4.

Table 1 Blood glucose, Body gain, HbA1C and Total Protein value were shown in three groups.

Groups	Body gain(g)	Blood glucose (mg/dL)			HbA_1C %
		Initial	Body gain 48 hours after experiment	End of study	
Control	6.2 ± 3	145 ± 15	156 ± 13	151 ± 12	5.7 ± 0.3
NTD group	-70.2 ± 7 [a]	148 ± 17	92.5 ± 21[a]	604.4 ± 48[a]	28.8 ± 1[a]
V_ETD group	-17 ± 12	144 ± 20	458 ± 28	380.3 ± 65[b]	4 ± 0.3[b]

a = $p < 0.05$ vs control group; b = $p < 0.05$ vs. NTD group.

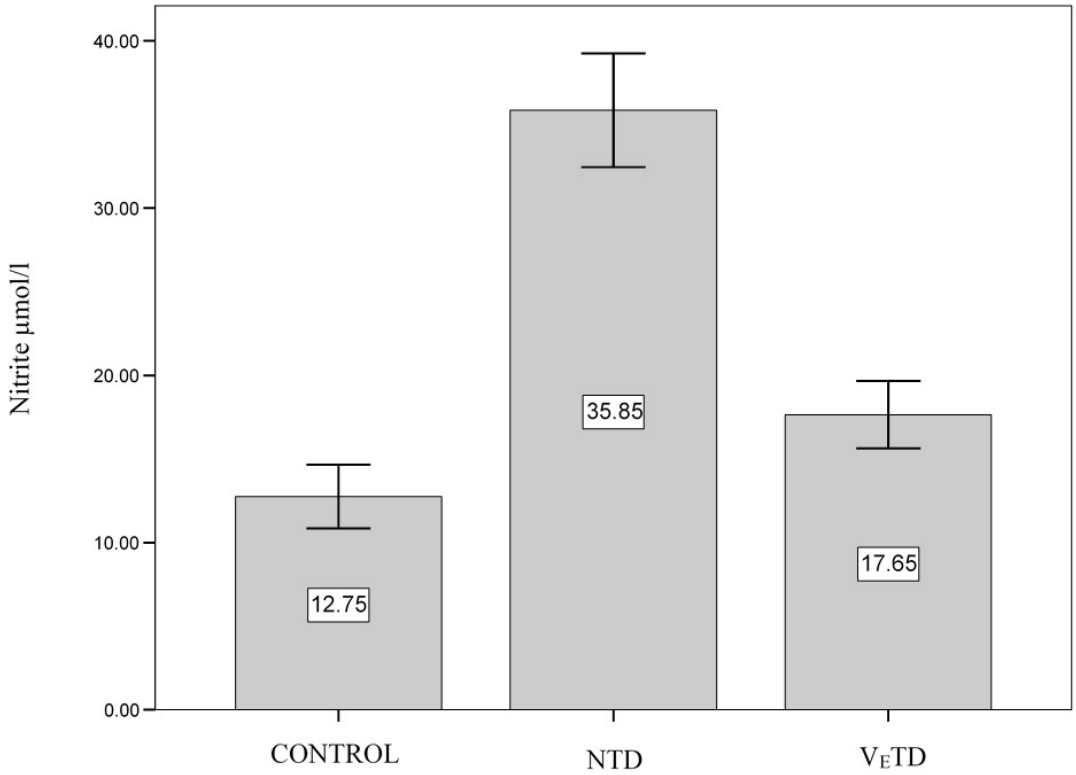

Fig. 1 Changes in NO_3^- content in control group, NTD (non-treated diabetic rats), V_ETD (vit E-treated diabetic rats) (Mean ± SD).

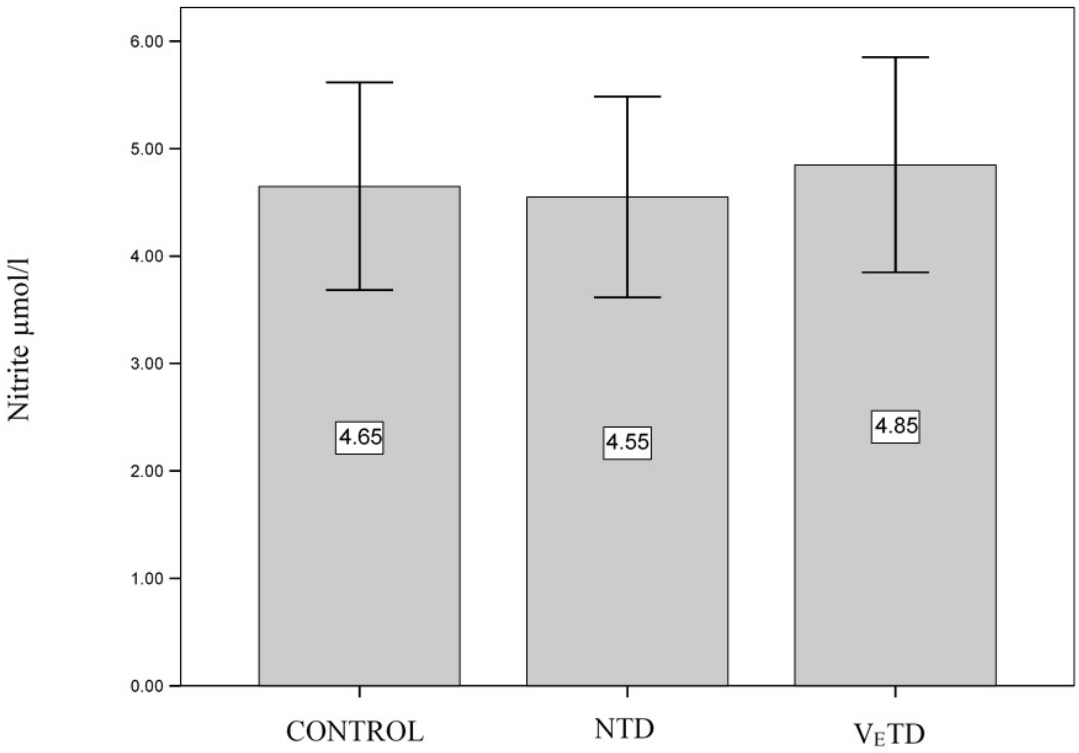

Fig. 2 Changes in NO_2^- content in control, NTD, V_ETD groups (Mean ± SD).

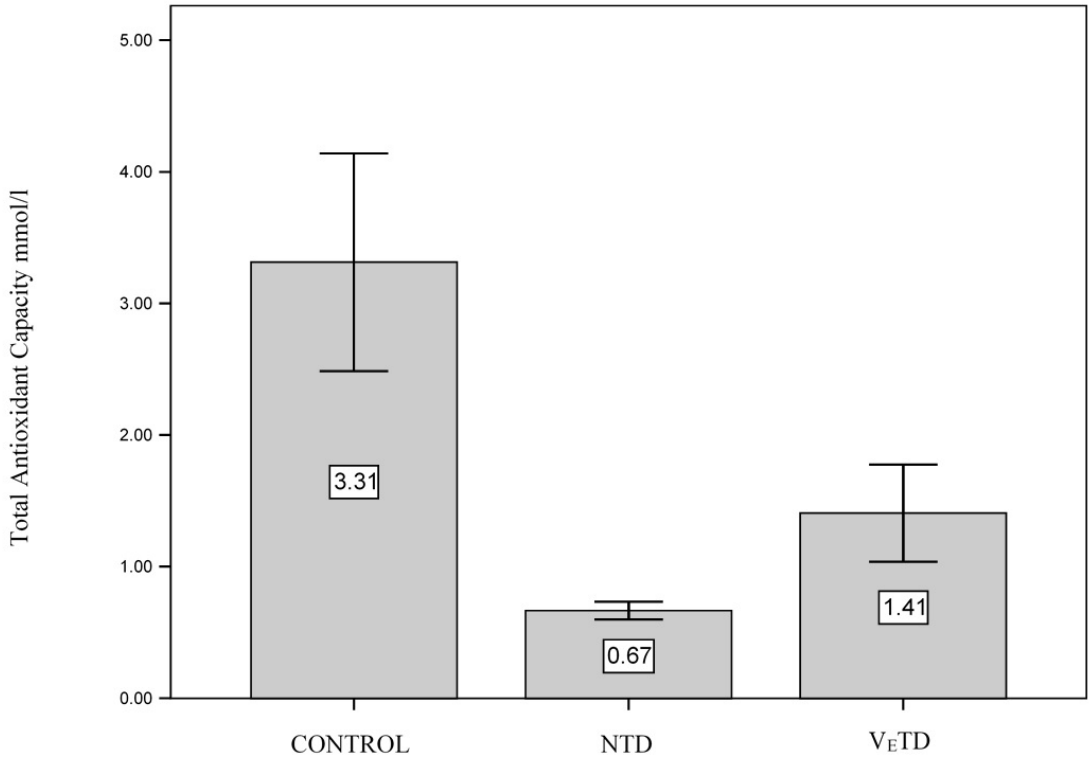

Fig. 3 Changes in TAC (total antioxidant capacity) content in control, NTD, V_ETD groups (Mean ± SD).

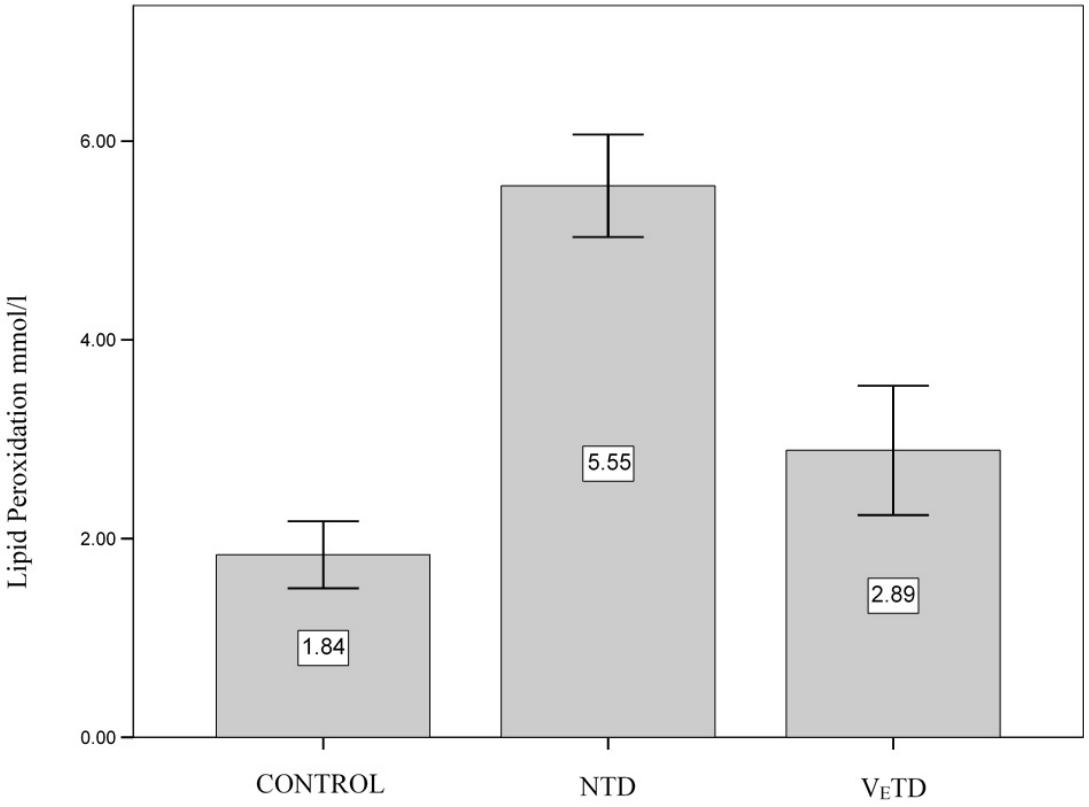

Fig. 4 Changes in LPO content in control, NTD (non-treated diabetic rats), V_ETD (vit E-treated diabetic rats) (Mean ± SD).

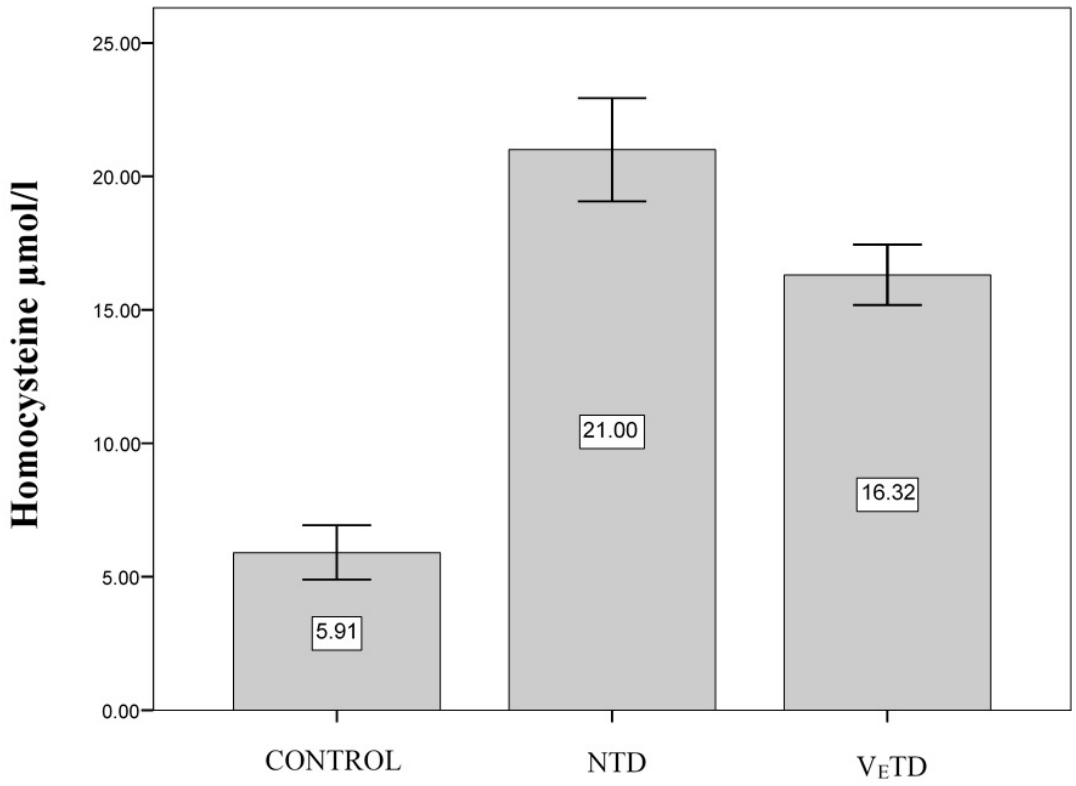

Fig. 5 Changes in homocysteine content in control, NTD, V_ETD groups (Mean ± SD).

Our research results indicate a significant increase in Hcy content in the NTD group compared the control group (21.00 ± 1.9 and 5.91 ± 1.0 µmol/L, respectively); $p < 0.05$. After treating with vit E, there was a significant decrease in Hcy content (16.32 ± 1.3 µmol/L) compared to diabetic rats; $p < 0.05$. However, significant increase was shown in the V_ETD group compared to the control group; $p < 0.05$, Fig. 5.

4. Discussion

Diabetes is a complex disease combining various other metabolic abnormalities. Increased cardiovascular disease is the major risk to cause morbidity and mortality in the complication of diabetes [1, 19]. Our major findings show that the contents of LPO, Hcy and NO_3^- have increased significantly in the heart of diabetic rats. These results are in agreement with other findings [20-22].

As we know, vit E is one of the fat-soluble vitamins and is considered as an important antioxidant. Vitamin E can react with free radicals, and avoid their reaction with other biomolecules, thus paralyzing their dangerous effects [16].

Our result shows that the amount of LPO in diabetic rats has increased. It is said that this increase occurs because of the increase in the production of free radicals in diabetic rats. The increase in the amount of free radicals is the result of an increase in the incidence of hyperglycemia [23].

We found that vit E has decreased the amount of LPO of cured diabetic rats twice more than of uncured diabetic rats (from 5.55 µmol/L NTD rats to 2.88 µmol/L in diabetic V_ETD rats). Also, an increase in oxidative stress may destroy beta cells and endothelial cells of the vascular tissue and cause a decrease of nitric acid activity in destroyed endothelial cells. Thus, more nitric oxide changes into nitrate and the amount of NO_3^- increases [24].

According to the study by Maree et al. [25] there is an increase in the amount of NO_3^- in diabetic rats, but it

was not an increase in the level of NO_2^-.

We also found that with the use of vit E, nitrite and nitrate levels of diabetic rats decrease perhaps because vit E affects nitric oxide and increases its efficiency by mixing it with free radicals [26].

Said et al. [27] showed that there is negative correlation between vitamin E and fasting blood glucose, LDL and triglyceride. Also they showed that Superoxide dismutase, Glutathione Peroxidase and vitamin E are important components in the cell defense against oxidative stress specially in patients with diabetic retinopathy. These findings according with our study that inhibition effects of vit E on Oxidative stress produced in diabetes patients.

Alper et al. [28] showed that there is an imbalance in oxidant/antioxidant status in the type-2 diabetes model of rat and vitamin supplementation improves the impairment in diabetic vasculature.

In this study, the amount of TAC in diabetic rat decreases. With an increase in the amount of the production of ROS in diabetic rats, the amount of antioxidants in the body decreases. TAC acts like an antioxidant and reacts with the ROS and, consequently, the amount of TAC decreases [29].

The reasons for increasing the Hcy amount are the changes that occurred in the enzymes during Hcy metabolism, and an increase of Hcy in the blood [22]. Also, this affects the main Hcy catabolism organs such as kidney and liver, changes their function, and decreases Hcy catabolism, leading to excretion of Hcy and an increase in the amount of Hcy in the blood [30].

Investigations conducted on the content of Hcy in diabetic rats are limited. Chico et al. [31] and Hultberg et al. [32] have proved that there is an increase of Hcy in diabetics. However, the research of Jacobs [30] shows that the amount of Hcy in diabetic rats decreased compared to the control group. The results of this study show that with the use of 300 mg of vit E, Hcy decreases in diabetic rats. The reason for this decrease might be the effects of vit E on produced ROS in hyperglycemia, and its effects on decreasing the amount of Hcy.

Most probably, the tested rats had high levels of glucose and had increased amounts of Hcy in the heart, a fact that can be a risk factor for heart problems related to diabetes mellitus [33, 34].

5. Conclusions

Our data suggest that increased Hcy, LPO and NO_3^- levels are common in diabetic patients and are as risk factors for heart failure and should be recognized as a cardiac risk factor in diabetic patients.

Patients with heart failure and documented high levels of LPO, NO_3^- and Hcy should be considered for therapy with vitamin E since treatment is effective, cheap, without risk, and may lower the risk of adverse cardiac events.

Vitamin E supplementation has a significant role in postponing the onset of the diabetic complications. Impaired diabetes and heart failure patients should in particular be screened since the incidence of LPO, NO_3^- and Hcy in these patients appears to be quite high.

Another issue of interest is the dose of vitamin E preparations. Determination of the most favored active doses of vitamin E requires further research. It seems that in the case of diabetic patients, doses of antioxidants should be higher than usual daily dose.

Conflict of Interest

We declare that there is no conflict of interest.

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