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Protective Effects of Vitamin E Against Motor Nerve Conduction Deficit in Diabetic Rats

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Abstract: The present study was done at Biology Department,Umm Al-Qura University,Saudi Arabia,during the period of December,2012 to April,2013.The study aimed to evaluate the abilityof vitamin E as an antioxidant to counteract the oxidative stress generated nerve complication, mainly nerve conduction velocity in diabetic rats. Rats were divided into 3 groups; control (G1), diabetic untreated rats (G2) and diabetic rats received 200 mg/kg bodyweight vitamin E daily by oral gavage (G3) for 6 weeks. Rats were rendered diabetic by intraperitoneal injection of streptozotocin (60 mg/kg body weight).Blood samples were taken at 2, 4 and 6weeks after the onset of diabetes for determination of plasma malondialdehyde level(MDA) and red cell superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase activities (GPx).At the end of 6th week,5 rats / group were dissected for Sciatic nerve - gastrocnemius muscle unit for assessment of nerve conduction velocity (NCV). Results revealed that diabetic rats exhibit significant higher levels of MDA allover the experimental times (4.70±0.55, 4.90±0.46, 5.50±0.56 Vs 3.30±0.40, 3.10± 0.45, 3.10±0.42), respectively. A significant increase in SOD activity was detected at 6th week of experiment (4.60± 0.18 Vs 2.45±0.06). Nerve conduction velocity was reduced significantly (1.55±0.09 Vs 4.20±0.42). Vitamin E supplementation significantly reduced MDA levels (1.30±0.09, 1.35±0.09, 1.20±0.08 Vs 4.70±0.55, 4.90±0.46, 5.50±0.56) and increased Gpx activity (0.32±0.03, 0.35±0.03, 0.37±0.04 Vs 0.13±0.01, 0.14±0.02, 0.13±0.01),respectively.Nerve conduction velocitywas significantly increased (3.95±0.28 Vs 1.55±0.09) as compared with diabetic untreated rats. In conclusions: vitamin E was able to counteracts the oxidative stress associating the diabetes mellitus maintaining normal motor nerve conduction velocity decreasing the risk of nerve complications that needs further clinical investigation.

Key words: Malondialdehyde · Antioxidant Enzymes · Vitamin E · Diabetic Rats · Nerve Conduction Velocity

INTRODUCTION

Oxidative stress has been implicated as playing a role in the development of diabetic complications such as a the rosclerosis [1], peripheral polyneuropathy, carcinogenesis [2] and increased incidence of abnormal embryonic development in diabetic mothers[3].Lipid peroxidation is a free radical-related process,which is potentially harmful because its uncontrolled,self-enhancing process causes disruption of membranes, lipids and other cell components[2].

The accumulating data suggest that in diabetes there is an increased production of reactive oxygen species[4] and decreased antioxidant scavenging activities [5]. This results in increased lipid peroxidation and DNA oxidation, as assessed by both elevated plasma levels of lipid and DNA oxidation products. There is a defensive mechanism consisting of antioxidant enzymes that play an important role in scavenging reactive oxygen species[6].

The organism's susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defenses. Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and lead to oxidative stress [7]. Diabetic polyneuropathy (DPN), the most common microvascular complication of diabetes mellitus, is a group of disorders that affecte both types of diabetic patients[8]. Many reports were available with regard to oxidative stress and antioxidant status of type 2 diabetic patients [9].

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Vitamin E is one of the most effective antioxidants in animals.It was shown that palm vitamin E extract increases the activity of the antioxidant enzyme glutathione peroxidase [10].The present study was planned to study the effects of Vit.E supplementation on plasma level of Malondialdehyde (MDA) and activities of antioxidant enzymes (Glutathione peroxidase / GPx, Superoxide dismutase / SOD and Catalase / CAT) in diabetic rats.The protective role of Vit.E on nerve function in diabetic rats was also investigated.

MATERIAL AND METHODS

Animals: Fifty Sprague-Dawley male rats of average body weight 200 g were used. Ten rats were checked for blood glucose level and used as normal control rats (G1). The remaining 40 rats were rendered diabetic. Diabetes was induced by an intraperitoneal injection of streptozotocin (Sigma, Watford, UK) in saline (60 mg/kg body weight) to an overnight fasted animal [10]. Fasting blood glucose was determined by using a glucometer and a reagent strip (Boehringer Mannheim, Mannheim, Germany) to confirm diabetes 2 days later. Thirty rats having blood glucose level exceeding 300 mg/dl were considered as diabetic rats and were divided into two equal groups (G2&G3). The rats were fed on normal standard food and water ad libitum.The treated group (G3)was given 200 mg/kg body weight α-tocopherol in olive oil by gavage daily. The untreated group (G2) received an equivalent volume of olive oil only.

Sampling and Techniques: Blood samples were taken at 2, 4 and 6 weeks after the onset of diabetes from rats of all experimental groups for determination of plasma level of glucose, MDA [11], activities of SOD [12], CAT [13] and GPx[14]in red cells. The institutional ethical committee approved the research procedures used in this study.

At the end of experimental time, dissection of sciatic nerve - gastrocnemius muscle unit preparation and the motor nerve conduction velocity (NCV) test were performed for 5 rats / group as described previously [15]. The animals were anesthetized with 30/2.5 mg/kg ketamine/xylazine to prevent discomfort. Body temperature was monitored with a dermal temperature probe and maintained at 32ºC with a warming lamp during NCV. Body temperature was maintained at 37ºC after NCV using a warming pad to ease animal stress from anesthetic. The nerve studies lasted less than 30 min per rat. The electrodes were cleaned with 70% alcohol between animals to maintain pathogen-free status.

Statistical Analysis: The obtained results were statistically analyzed using ANOVA test followed by Student's t-test. P-values less than.05 were considered to be significant.

RESULTS

Table (1) illustrates results of plasma glucose, MDA, red cell enzymes activities of Gpx, SOD, CAT and NCV. Comparing with the control rats (G1), data indicates that diabetic rats (G2) exhibited a significant elevations in the plasma MDA level allover the experimental times (4.70±0.55, 4.90±0.46, 5.50±0.56 Vs 3.30±0.40, 3.10± 0.45, 3.10±0.42), respectively. Also, a significant increase in SOD activity was recorded at 6 weeks of experiment (4.60± 0.18 Vs 2.45±0.06). No significant alterations were recorded in GPx or CAT activities allover the experimental times.In addition, NCV was found to be reduced significantly (1.55±0.09 Vs 4.20±0.42)whichwas detected at 6thweek of experiment.

Table 1: Blood glucose level, activities of MAD, GPx,CAT,SODand nerve conduction velocity of normal and diabetic rat groups

	Time								
	2 weeks			4 weeks			6 weeks		
Parameters	G1	G2	G3	G1	G2	G3	G1	G2	G3
Glucose */mg/dl*	98.00±4.40^{a}	250.00±6.20^{b}	280.00±6.90^{c}	100.00±4.80^{a}	310.00±7.50^{d}	326.00±8.00^{d}	102.00±5.00^{a}	356.00±8.12^{f}	360.00±8.23^{f}
MAD*/nmol/ml*	3.15±0.40^{a}	4.80±0.55^{b}	1.30±0.09^{c}	3.10±0.45^{a}	4.90±0.46^{b}	1.35±0.09^{c}	3.10±0.42^{a}	5.50±0.56^{b}	1.20±0.08^{c}
GPx*/activity/mg Hb*	0.16±0.02^{a}	0.13±0.01^{a}	0.32±0.03^{b}	0.17±0.02^{a}	0.14±0.02^{a}	0.35±0.03^{b}	0.15±0.02^{a}	0.13±0.01^{a}	0.37±0.04^{b}
CAT */activity/mg Hb*	0.32±0.05	0.33±0.04	0.35±0.04	0.32±0.05	0.34 ±0.05	0.32±0.04	0.33±0.04	0.37±0.06	0.35±0.05
SOD*/activity/mg Hb*	2.45±0.07	2.75±0.07	2.50±0.05	2.60±0.07	2.75±0.08	2.80 ±0.08	2.45±0.06^{a}	4.60±0.18^{b}	4.80±0.22^{b}
NCV */m/ Sec.*	-	-	-	-	-	-	4.20±0.42^{a}	1.55±0.09^{b}	3.95±0.28^{a}

-Values are expressed as Means±SE, means at the same raw having different letters are significantly different from each other at P <. 05. G1: Control rats, G2: Diabetic untreated rats, G3: Diabetic treated rats.MAD: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione Peroxidase, CAT: Catalase, NCV: Nerve conduction velocity.

Comparable with rats of G2, table (1) shows that supplementation with Vit.E (G3) was associated with a significant decrease in MDA level (1.30±0.09, 1.35±0.09, 1.20±0.08 Vs 4.70±0.55, 4.90±0.46, 5.50±0.56) and significant increase in GPx activity (0.32±0.03, 0.35±0.03, 0.37±0.04 Vs 0.13±0.01, 0.14±0.02, 0.13±0.01), respectively allover the experimental times. Meanwhile, SOD activity was found to be significantly increased (4.80±0.22 Vs 2.45±0.06) towards the later stage of experiment as compared with rats of G1. In addition, NCV was recorded to be significantly increased (3.95±0.28 Vs 1.55±0.09) approaching the control level (4.20±0.42).

DISCUSSION

Several experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Free radicals are formed in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and several damages in diabetes mellitus [10].

In the present study, the MDA levels, a lipid peroxidation product and a marker of oxidative stress, were decreased significantly in treated diabetic rats as compared to control or untreated diabetic rats.This clearly shows that diabetic untreated rats were exposed to an increased oxidative stress via lipid peroxidation. Other researchers have also reported elevated lipid peroxidation products in blood samples of type 1 and 2 diabetic patients[6,16,17]. Abnormally-high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes. Antioxidant enzyme-dependent defenses play an important role in scavenging free radicals produced under oxidative stress [18].

Plasma MDA levels were increased in untreated diabetic rats. This probably reflects the increase in lipid oxidation due to either increased production of free oxidative radicals [4] or decreased antioxidant defence mechanisms [5] or both. The same observations have also been reported in humans [19] and rats [10]. Supplementation with Vit.E significantly decreased the level of MDA in treated diabetic groups, confirming its role as a powerful antioxidant. Similar observations were also noted with the use of palm vitamin E extract [10].

Results from the antioxidant enzymes determinations showed that basal GPx levels are lower in diabetic rats, confirming the earlier reports[5,20]. Oral supplementation with α-tocopherol increased the level of GPx. This enzyme scavenge free radicals and prevent oxidative damage [21].This finding is slightly different to what was observed earlier by supplementing palm vitamin E extract to normal and diabetic patients[10].

Current data revealed also that glutathione peroxidase, an antioxidant, of untreated diabetic rats were significantly low indicating decreased scavenging capacity of glutathione-dependent antioxidant defensive system against elevated lipid peroxidation processes. Diabetic humans have shown increased lipid peroxidation and decreased levels of glutathione peroxidase [22].

No significant changes in superoxide dismutase (SOD) activity were observed except at the end of 6 weeks in treated diabetic rats. Previous studies has shown that no significant changes in the SOD activity are recorded in type 2 diabetic patients [23].Oral daily supplementation with Vit.E was found to increase the level of SOD in diabetic rats [10]. The differences in SOD responses might be due to the differences in doses of Vit.E used.

In the present study,CAT activity was shown to be constant in all experimental groups. These results agree well with that of Musalmah *et al.* [10] found that supplementation of palm vitamin E extract does not change the activity of CAT enzyme that remained constant in both the α-tocopherol-treated and untreated rats. CAT is the slowest of the above antioxidant enzymes to respond to an increased level of free radicals in blood. Therefore, the time interval through which this experiment was carried out may be insufficient to witness any change in the activity of this enzyme.

Further more, the diabetic rats exhibited nerve conduction velocity (NCV) deficits at the end of the 6^{th} week of experiment.Supplementation with vitamin E was associated with an improvement in NCV. It was reported that diabetic patients were exposed to an increased oxidative stress via lipid peroxidation and the free radicals may play an important role in the development of complications [22]. Moreover, studies suggested that the increase in lipid peroxidation and the decline in antioxidant defenses may appear early in diabetes mellitus patients, before the development of secondary complications [10].The protective role of vitamin E supplementation may be attributed to its powerful antioxidant properties that could minimize the onset of oxidative stress in diabetic rats protecting them against the NCV deficits.

In conclusion, the present study showed that vitamin E enhances the antioxidative stress mechanisms in diabetic rats decreasing the risk of nerve complications that needs a further clinical investigation.

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