Original Article

Exploring the Impact of Antioxidants on Nerve Health and Oxidative Stress in Diabetic Neuropathy

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Abstract

Background: Persistent hyperglycemia supports the occurrence of oxidative stress and generation of endogenous free radicals, which subsequently triggers the development of diabetes complications such as peripheral neuropathy. The neurons and Schwann cells are especially susceptible to glycolytic damage due to the change in glucose levels. It has also proposed antioxidants, such as vitamins E and C, to reduce the development of neuropathy and the nerve conduction velocity (NCV).

Objective: The aim of the current study was to assess the consequences of antioxidant supplement on a model of diabetic rats and to investigate the effects of antioxidant supplement on NCV.

Methods: Diabetes Diabetes was induced in rats by streptozotocin (STZ) intraperitoneal injection. The animals were then divided into six aspects of experimentation. Measurements of blood glucose and anthropometric values were taken. Isolated nerves, NCV was recorded. One-way ANOVA was used to analyze the data and the Bonferonni post-hoc test was carried out.

Results: The level of glycaemic in diabetic rats increased (254mg^{-}1dL); but with the vitamin E and C, the level dropped to 229mg/dl and 206mg/dl respectively. Supplementation with vitamin E relieved the NCV deficits in diabetic rats but vitamin C did not achieve any statistically significant effect on NCV.

Conclusion: Vitamin E Supplementation enhances nerve conduction in diabetic rats, indicating that it may be used to treat diabetic neuropathy.

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Introduction

Diabetes mellitus is a fast-growing international health issue, the impact of which is enormous in regard to morbidity and mortality. Epidemiological data suggest that the population is estimated to have been afflicted by millions of people around the globe with particularly high rates in low and middle income nations. As an example, over 80,000 women and 36,000 men are dying each year in Pakistan due to diabetes-related problems with a disproportionate involvement of the urban populations (12.21%) compared to the rural ones

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(7.0%). There are Type 1 and Type 2 types of the disease, but the less common variants form a spectrum. Diabetic peripheral neuropathy (DN) is one of the most prevalent among its serious complications, afflicting about half of patients with diabetes; it is a bilateral, symmetric nerve damage, either small or large fibers.[1-5]

Diabetic neuropathy pathophysiology is multifactorial, with metabolic perturbation caused by chronic hyperglycemia. One of the most crucial factors is increased oxidative stress due to the excess of free radicals which impairs neuronal structure and reduces the conduction of axons. High glycemic levels contribute to the generation of reactive oxygen species (ROS) and advanced glycation end products (AGEs) and lead to signaling cascades such as NF- -B, polyol pathway, and protein kinase C, the aggregate outcome of which is peripheral nerve ischemia, mitochondrial dysfunction, and axonal degeneration [T4-T6].[6-8]

Oxidative stress is generally considered to be a key pathogenic determinant in the development of diabetic neuropathy, and in this respect, antioxidants have received a significant amount of academic attention as potential treatment agents. These compounds have effects of counteracting reactive oxygen species as well as increasing intrinsic antioxidant defense systems. Special focus is put on vitamins E and C which were reported repeatedly to have protective effects. Vitamin E - a lipid-soluble chain-breaking antioxidant found in green leafy vegetables has the effect of inhibiting lipid peroxidation, as well as, enhancing the sensitivity of insulin. Conversely, the water-soluble antioxidant vitamin C, which is abundant in citrus fruits and other vegetables, protects against oxidative damage by scavenging free radicals, decreasing protein glycation, and also through synergistic protection against oxidative damage by regenerating oxidized vitamin E.[9-14]

Despite all the studies conducted on oxidative stress in diabetic complications, there exists a gap on the effect of concomitant administering vitamin E and vitamin C on nerve conduction parameters in diabetic neuropathy.[15,16] Many studies focus on biochemical biomarkers but the data on functional neurophysiological outcomes especially nerve conduction velocity is limited. Sealing this gap might help in the recognition of simple, practical intervention that are geared towards preventing or reducing the development of neuropathic disorders.[17-19]

This study aims at exploring the effects of vitamins C and E on nerve conduction velocity in one of the diabetic models based on the hypothesis that the use of antioxidant supplements could reverse neuropathic injury and improve neurophysiological performance in diabetic neuropathy.

Methods

The animal house of the Panjwani Centre of Molecular Medicine Karachi University purchased a cohort of thirty albino rats (age 1213 weeks; body weight 200250g) used as a source of experimental animals. All the experimental procedures were in line with the ICCBS ethics. After the period of one week acclimatization, the subjects were assigned to six experimental groups of four animals each:

- **Group 1:** Control receiving normal saline
- **Group 2:** Control receiving Vitamin E (500 mg/ kg/ day)
- **Group 3:** Control receiving Vitamin C (500 mg/ kg/day)
- **Group 4:** Diabetic, induced with Streptozotocin (STZ)
- **Group 5:** Diabetic + Vitamin E (500 mg/kg/day)
- **Group 6:** Diabetic + Vitamin C (500 mg/kg/day)

Induction of Diabetes: Diabetes was induced in Groups 4–6 via a single intraperitoneal injection of STZ at 60 mg/kg, dissolved in citrate buffer (pH 4.5). Blood glucose levels were measured 10 days after injection; those with a level that was above 200mg/dL were considered diabetic. Treatment regimen Vitamin E and vitamin C were administered orally through gavage at the rate of 500 mg/kg/day/28-day as a continuous course starting a day after the assessment of diabetic conditions was made. Assessments: Once the treatment regimen was over, the animals were weighed and the concentration of blood glucose was re-assessed. Rats were then euthanized, and the sciatic nerve was excised. Nerve Conduction Velocity (NCV) recording the excised nerve segments were placed in Krebs buffer and mounted in a nerve chamber with standardized electrodes (ground, stimulation, and recording electrodes). The nerve was positioned over a 4 cm distance between the electrodes. NCV was recorded at 0, 15, 30, 45, and 60 minutes using a PowerLab data acquisition system (AD Instruments).

Data Analysis were expressed as Mean ± Standard Error. Statistical significance was evaluated using one-way ANOVA with post hoc testing; $p < 0.05$ was considered significant.

Results

Streptozotocin (STZ) successfully induced diabetes in rats as demonstrated by a significantly higher fasting

blood glucose (FBG) levels in diabetic group compared to controls (FBG in control: 104.3 ± 23.9, STZ rats: 254.0 ± 23.9. Glucose levels in STZ supplemented with Vitamin E were: 229.5 ± 22.5 and STZ supplemented with Vitamin C: were 206.5 ± 29.5mg/dl. However, the FBG levels of STZ induced diabetic rats supplemented with Vitamin E and vitamin C were reduced but not statistically significant (p=1.000), (p=0.069) respectively (Table 1).

Table 1: *Fasting blood glucose of rats, 21 days following induction of diabetes*

	Control (n=4)	STZ induced diabetics (n=4)	Diabetics + Vitamin E (n=4)	Diabetics + Vitamin C (n=4)
Fasting blood glucose (mg/dl)	104.3 ± 23.9	254.0 ± 23.9	229.5 ± 22.5	206.5 ± 29.5
Control		p<0.01	p<0.01	p<0.01
STZ induced diabetics	p<0.01		p=1.000	p=0.069

P<0.05 is significant. Values are shown as mean ± SD

NCV was studied using right and left sciatic nerve over 15 minute intervals for an hour in 4 groups – control group, diabetic group, diabetic group supplemented with Vitamin C, and diabetic group supplemented with Vitamin E.

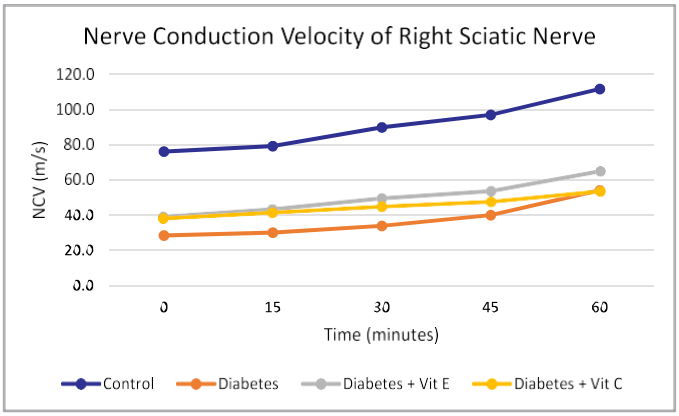

Figure 1: *Comparison of nerve conduction velocities of right sciatic nerve in control group (blue), diabetic group (orange), diabetic group supplemented with Vitamin C (yellow) and diabetic group supplemented with Vitamin E (gray) at 0, 15, 30, 45 and 60 minute time intervals*

There was a significant reduction in NCV in diabetic

Table 2: *Nerve Conduction Velocity of right sciatic nerve at 0, 15, 30, 45 and 60 minute time intervals*

		NCV (m/s)				
		0min	15mins	30mins	45mins	60mins
Control	N ± sd	76.2 ± 8.4	82.1 ± 14.6	89.9 ± 8.1	97 ± 9.5	111.8 ± 10.2
Diabetes	N ± sd	28.4 ± 3.6	30.0 ± 4.2	33.8 ± 3.2	39.9 ± 3.8	54.0 ± 15.14
	p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Diabetes & Vitamin C	N ± sd	38.6 ± 2.4	41.8 ± 2.3	45.1 ± 3.5	47.8 ± 3.3	53.5 ± 4.4
	p-value	0.494	1.000	0.071	0.964	1.000
Diabetes & Vitamin E	N ± sd	39.5 ± 7.6	43.6 ± 6.3	49.7 ± 5.1	53.8 ± 5.8	64.8 ± 14.0
	p-value	0.321	0.102	0.004*	0.04*	1.000

p-value of diabetic control is compared to controls; p -value of Diabetes + Vitamin C and Diabetes + Vitamin C is compared to Diabetes group p<0.05 is significant

Table 3: *Nerve Conduction Velocity of left sciatic nerve at 0, 15, 30, 45 and 60 minute time intervals*

		NCV (m/s)				
		0min	15mins	30mins	45mins	60mins
Control	N ± sd	81.7 ± 7.0	83.9 ± 8.1	89.4 ± 7.9	96.3 ± 12.0	113.1 ± 13.7
Diabetes	N ± sd	27.0 ± 5.0	31.0 ± 4.3	35.6 ± 3.1	40.5 ± 2.7	48.6 ± 5.0
	p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Diabetes & Vitamin C	N ± sd	37.1 ± 1.8	39.6 ± 4.4	42.4 ± 3.6	47.6 ± 1.8	53.6 ± 4.4
	p-value	0.218	0.896	1.000	1.000	1.000
Diabetes & Vitamin E	N ± sd	39.8 ± 7.4	42.2 ± 7.6	48.0 ± 5.3	52.6 ± 7.0	65.8 ± 10.6
	p-value	0.045*	0.266	0.087	0.323	0.142

P-value of diabetic control is compared to controls; p -value of Diabetes + Vitamin C and Diabetes + Vitamin C is compared to Diabetes group p<0.05 is significant.

group compared to the control group at every time point ranging from 0mins (control: 76.2 ± 8.4 m/s DM: 28.4 ± 3.6 m/s, p<0.001) to 15mins (control: 82.1 ± 14.6 m/s DM: 30.0 ± 4.2 m/s, p<0.001), 30mins (control: 89.9 ± 8.1 m/s DM: 33.8 ± 3.2 m/s, p<0.001), 45 mins (control: 97 ± 9.5 m/s DM: 39.9 ± 3.8 m/s, p<0.001) to 60mins (control: 111.8 ± 10.2 m/s DM: 54.0 ± 15.14 m/s, p< 0.001) in the right sciatic nerve.

An improvement was noticed at 30mins (DM: 33.8 ± 3.2 m/s DM & Vit E: 49.7 ± 5.1 m/s, p=0.004) and 45mins (DM: 39.9 ± 3.8 m/s DM & Vit E: 53.8 ± 5.8 m/s, p=0.04) in the diabetic group supplemented with Vitamin E. However, supplementation with Vitamin C after induction of diabetes did not yield any statistically significant improvement in NCV (Table 2, Figure 1).

There was a significant reduction in NCV in diabetic group compared to the control group at every time point ranging from 0 mins (control: 81.7 ± 7.0 m/s DM: 27.0 ± 5.0 m/s, p<0.001) to 15mins (control: 83.9 ± 8.1 m/s DM: 31.0 ± 4.3 m/s, p<0.001), 30mins (control: 89.4 ± 7.9 m/s DM: 35.6 ± 3.1 m/s, p<0.001), 45 mins (control: 96.3 ± 12.0 m/s DM: 40.5 ± 2.7 m/s, p<0.001) to 60mins (control: 113.1 ± 13.7 m/s DM: 48.6 ± 5.0 m/s, p< 0.001) in the left sciatic nerve.

At 0mins, there was an improvement noticed in nerve conduction velocity of diabetic mice treated with Vitamin E (DM: 27.0 ± 5.0 m/s DM & Vitamin E: 39.8 ± 7.4 m/s, p=0.045. By and large, no statistically significant changes were noted in the nerve conduction velocity other than 0 min readings of diabetic mice supplemented with either Vitamin E or Vitamin C (Table 3, Figure 2).

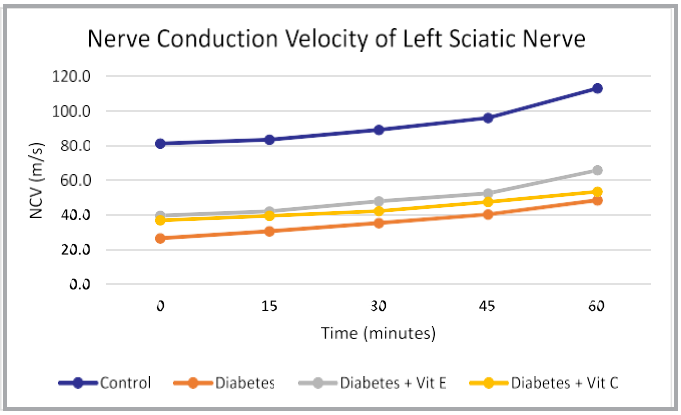

Figure 2: *Comparison of nerve conduction velocities of left sciatic nerve in control group (blue), diabetic group (orange), diabetic group supplemented with Vitamin C (yellow) and diabetic group supplemented with Vitamin E (gray) at 0, 15, 30, 45 and 60 minute time intervals*

Discussion

This study demonstrated that Vitamin E supplementation significantly improved nerve conduction velocity (NCV) in diabetic rats, indicating a neuroprotective effect against diabetic neuropathy. These findings align with previous research showing that Vitamin E, owing to its lipophilic antioxidant properties, helps preserve nerve function by reducing oxidative stress and supporting myelin integrity.[1,2,13,17] Supplementation with vitamin C on the contrary, did not provide statistically significant improvements in nerve conduction velocity throughout the experiment, which can be attributed to the hydrophilic nature, limited cellular absorption during hyperglycemic conditions, or dose-dependent actions.[12,13]

The most common way Vitamin E protects the brain seems to be in the form of the scavenging of free radicals thus preventing lipid peroxidation and maintaining the structural integrity of neuronal membranes and myelin sheaths.[16-18,20-22] and could also help in the regeneration of nerve fibers by protecting Schwann cells and promoting cellular repair.[17-19] As an antioxidant, Vitamin C is water-soluble and neutralizes oxidative stress by counterbalancing free radicals and restoring Vitamin E; however, its effect might be limited by the dosage used in the present study, which might be due to the limitation of their cellular absorption in the state of diabetes.[12,21] What is more, when the Vitamin C doses are large, it autoxidizes at a higher concentration and thus reduces its antioxidant effect.[23]

There are a number of limitations to consider. This small sample was also a probable limitation to the statistical power to identify minor effects, particularly in Vitamin C. The fact that high antioxidant doses are used also makes one concerned about the possibility of toxicity or pro-oxidant effects at higher doses.[23-25] Also, the study was conducted over a period of 21 weeks, which was not enough to measure long-term nerve structural changes or regeneration. The lack of histopathological evaluations will not allow direct correlation of functional and morphological repair of the nerves.

Further studies need to consider larger samples, dose importance analyses and follow-up to validate and elaborate such results. Histological studies of nerve tissues into the study can be useful in explaining morphological counterparts of functional recovery. Notably, to apply these results to clinical practices, it is essential to evaluate the optimal dosing and safety of antioxidants, and the possibility of coming up with an adjunctive treatment of diabetic neuropathy.[12,17,19]

Conclusion

The research shows that Vitamin E plays a great role in enhancing nerve conduction velocity in diabetic rats, and therefore it could be used as a neuroprotective agent in diabetic neuropathy. The future studies should aim on larger sample sizes, prolonged dose and clinical trials to confirm its effectiveness and safety in human beings.

Ethical Approval: The Institutional Bioethical Committee, University of Karachi approved this study vide IBC KU-390/2024.

Conflict of Interest: The authors declare no conflict of interest.

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Authors' Contribution

SA: Conception & design, acquisition of data, critical revisions for important intellectual content

SK: Conception & design, drafting of article, final approval of the version to be published

SJ: Analysis & interpretation, critical revisions for important intellectual content

FS: Analysis & interpretation, critical revisions for important intellectual content

NJ: Drafting of article

AS: Critical revisions for important intellectual content, final approval of the version to be published

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