PROTECTIVE INFLUENCE OF VITAMIN E ON THE ANTIOXIDANT DEFENCE SYSTEM IN THE WHOLE BLOOD AND LIVER OF NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The effect of oral administration of vitamin E for twenty-eight consecutive days on blood glucose, reduced glutathione levels, antioxidant enzymes (superoxide dismutase and catalase activities), and levels of malondialdehyde (as an index of free radical-mediated lipid peroxidation) was observed in the whole blood and liver of normal and alloxan-induced diabetic rats. It was found that oral administration of vitamin E significantly ($p < 0.05$) lowered the blood glucose level and increased the body weight of the diabetic rats. The activities of superoxide dismutase and levels of reduced glutathione increased significantly ($p < 0.05$) while the level of lipid peroxidation decreased.

KEY WORDS

Vitamin E, Antioxidant enzymes, Diabetes, Reduced glutathione, Lipid Peroxidation.

INTRODUCTION

Oxidative stress lies at the root of diseases such as cancer, atherosclerosis, rheumatic arthritis, hematological and neurodegenerative disorders (1, 2), with more making the list among which is diabetes mellitus.

Oxidative stress is the imbalance between the generation of reactive oxygen species and the activity of the antioxidant defenses. Severe oxidative stress can cause cell damage and death and it has been implicated in numerous human diseases including cancer, diabetes, malaria and atherosclerosis (3). Nowadays diabetic micro and macroangiopathy are considered to be polyetiological, multifactorial diseases where persistent hyperglycemia plays the leading part (4). Hyperglycemic effects are reinforced additionally by reactive oxygen types formed in the course of non-enzyme glycosylising of proteins and glucose auto oxidation occurring simultaneously (5).

Diabetes mellitus is one of the pathological conditions that are always accompanied by oxidative stress i.e. with the

preponderance of oxidative reactions over the antioxidative protection of tissues (6). Increased production of reactive forms of oxygen and lipoperoxides together with lowered levels of substances (vitamin C, vitamin A, lipoic acid, glutathione) having antioxidative reaction and enzymes acting as antioxidative agents such as superoxide dismutase and catalase can be found in the blood and tissues of patients affected with the disease (7). According to the current views, the antioxidative stress plays a significant role in the etiopathogenesis of diabetes mellitus of both first and second types and has key importance in the occurrence and development of diabetic complications (8).

Damage to cells caused by free radicals is believed to play central role in the ageing process and in disease progression. Advanced glycation or glycosylation end products (AGEs) are the products of glycation and oxidation (glycoxidation), which are increased with age and at an accelerated rate in diabetes mellitus (9, 10). In vitro studies have suggested that glycation itself may result in production of superoxide (11, 12). Oxidation has been hypothesized to result in generation of superoxide, H_2O_2 and through transition metal catalysis, hydroxyl radicals (13). Excess of reactive oxygen types contributes to intensive peroxidation of plasma lipids and so plays decisive role in formation of atheromatous plaque. Initially LDL accumulates in extra cellular space of small arteries walls and is oxygenated. There are formed the so-called minimum modified LDL (14). Antioxidants are our first line of defense against free radical

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damage. The need for antioxidants become even more critical with increased exposure to free radicals. Pollution, cigarette smoking, drug, illness, stress and even oxidative stress can increase free radical exposure (15).

Vitamin E, a major lipid-soluble antioxidant is the most effective chain-breaking antioxidant within the cell membrane, where it protects membrane fatty acids from lipid peroxidation (16). Vitamin E decreases hepatic fibrosis, bile ductal proliferation and inflammatory infiltration. The combined therapy of vitamin E and selenium decrease hepatocellular damage and these antioxidant agents have protective effect on hepatocytes (17). Vitamin E also prevents leakage of enzymes out of cells and into circulation and its supplementation was purported to reduce lipid peroxidation in plasma, skeletal muscle, heart and liver (18). Recent evidence suggests that vitamin E enhances glutathione levels and may play a protective role in magnesium deficiency-induced cardiac lesions.

The present study was carried out to investigate the comparative effects of vitamin E on the endogenous antioxidant status of both whole blood and liver, normal and diseased status and to monitor the effects of vitamin E on assayed parameters in dose dependent manner.

MATERIALS AND METHODS

Sixty albino rats were used as experimental subjects and they were obtained from the animal house of the Department of Physiology, University of Ibadan, Nigeria, housed in well-ventilated cages and fed on growers mash for four weeks. The animals (average weight 180g) were grouped into ten, each group containing six animals. Diabetes was induced intraperitoneally by a single dose of alloxan at a concentration of 150mg/kg body weight after a twenty-four hour fasting. Forty-

eight hours after the administration of alloxan, fasting blood glucose concentration was determined by glucose oxidase method using glucometer with glucose test strip (One Touch Basic). Animals having blood glucose concentration of over 250mg/dl were considered diabetic and selected for use.

Administration of drug : Di- α -tocopheryl acetate was used as the source of vitamin E (a product of ga- pharmaceuticals, 5A.46 Agissilaon street, 17341, Athens, Greece) in four different concentrations (in mg) : 50 ,100,200 and 400. Diabetic and normal control groups (DC and NC) were given clean water while vitamin E was suspended in water and administered orally to the test groups. The animals were placed on treatment for twenty-eight consecutive days.

Tissue preparation : After twenty-eight days, the animals were sacrificed through cervical dislocation, quickly decapitated and blood were collected from their hearts into lithium heparinized tubes, swirled very well and kept until used. Liver tissues were quickly removed, trimmed to remove excess tissue, blotted with tissue paper chopped and homogenized in 0.25M sucrose solution. These were centrifuged at 5,000 rpm twice and the supernatant was kept prior analysis.

Enzyme Assays : Catalase activity was assayed by the method of Sinha (19). Superoxide dismutase activity was estimated by the method of Misra and Fridovich (20). The level of reduced glutathione was determined using the method of Ellman (21). Free radical mediated damage was assessed by measurement of the extent of lipid peroxidation in terms of malondialdehyde (MDA) formed, essentially according to the method of Wills (22). Protein was determined by the method of Lowry *et al.* (23). Student's 't'-test was employed for statistical analysis.

RESULTS

Table 1: Changes in body weight (g) of rats treated with vitamin E

	Normal Control	50IU	100IU	200IU	400IU	Diabetic control	50IU	100IU	200IU	400IU
Week 1	133 $\pm$ 7.1	111 $\pm$ 5.3 ^b	90 $\pm$ 11.8 ^b	84 $\pm$ 6.3 ^b	72 $\pm$ 4.6 ^b	195 $\pm$ 2.4	187 $\pm$ 11.3 ^b	149 $\pm$ 11.8 ^b	160 $\pm$ 4.8 ^b	245 $\pm$ 9.0
Week 2	137 $\pm$ 7.6	109 $\pm$ 5.7 ^b	102 $\pm$ 5.2 ^b	94 $\pm$ 4.2 ^b	78 $\pm$ 3.8 ^b	178 $\pm$ 7.7	149 $\pm$ 2.9 ^b	141 $\pm$ 12.6 ^b	108 $\pm$ 5.1	249 $\pm$ 6.9
Week 3	155 $\pm$ 6.0	110 $\pm$ 5.5 ^b	112 $\pm$ 8.7 ^b	104 $\pm$ 6.8 ^b	87 $\pm$ 1.0 ^b	161 $\pm$ 4.5	186 $\pm$ 9.0	143 $\pm$ 9.0	138 $\pm$ 8.3 ^b	241 $\pm$ 6.8
Week 4	163 $\pm$ 6.2	110 $\pm$ 5.5 ^b	132 $\pm$ 6.9 ^b	122 $\pm$ 8.1 ^b	86 $\pm$ 3.5 ^b	152 $\pm$ 2.0	174 $\pm$ 5.0	163 $\pm$ 6.1	135 $\pm$ 9.4	233 $\pm$ 5.5

Values are Mean $\pm$ SD; Numbers with superscript are significantly different ($p < 0.05$) from control.

There were noticeable and significant ($p < 0.05$) changes in body weights of the animals treated with vitamin E. The change was more pronounced in the normal treated rats.

Table 2: Blood glucose concentration (mg/dl) of diabetic rats treated with vitamin E

	Diabetic Control	50IU	100IU	200IU	400IU
Week 1	268±19.30	275±14.85	388±16.44	393±16.13	390±23.50
Week 2	213±3.89	202±6.29	76±2.97 ^b	267±19.67	322±2.82
Week 3	202±7.45	206±0.00	78±3.46 ^b	97±4.38	257±9.19
Week 4	202±7.45	184±0.00	78±3.46 ^b	90±8.77	183±8.50

Values are Mean ±SD; Numbers with superscript are significantly different (p<0.05) from control. There was also significant (p<0.05) change in the blood glucose concentration of the diabetic treated rats.

Table 3: Effect of Vitamin E on superoxide dismutase activity in normal and diabetic rats

	Normal Control	50IU	100IU	200IU	400IU	Diabetic control	50IU	100IU	200IU	400IU
Blood	0.773±0.30	0.767±0.0	1.334±0.12	1.733±0.00 ^a	2.184±0.04	0.500±0.04	0.600±0.04	0.700±0.02 ^b	0.800±0.00	0.820±0.00
Liver	0.316±0.02	0.333±0.02	0.600±0.05	0.844±0.05 ^a	0.833±0.00	0.733±0.00	0.767±0.00	0.867±0.05 ^b	0.833±0.30	0.890±0.00

Values are Mean ± SD; Numbers with superscripts are significantly different (p<0.05) from control.

There was an increase in the activity of superoxide dismutase (SOD) in the whole blood of treated animals in a concentration dependent manner when compared with the controls. Reduction in whole SOD activity was noted in the diabetic control group than in the normal untreated control. Similar result was obtained in the activity of the SOD in the liver but the activity of the enzyme increased in the blood more than it did in the liver.

Table 4: Effect of Vitamin E on catalase activity in normal and diabetic rats

	Normal Control	50IU	100IU	200IU	400IU	Diabetic control	50IU	100IU	200IU	400IU
Blood	0.180±0.00	0.410±0.03	0.420±0.00	0.520±0.00	0.580±0.04	0.170±0.04	0.180±0.18	0.260±0.18	0.280±0.00	0.360±0.04
Liver	0.680±0.00	0.780±0.47	1.000±0.00	1.100±0.51	2.670±0.00	0.610±0.00	1.290±0.00	2.940±0.52	3.420±0.00	4.260±0.00

Values are Mean ± SD

As shown in Table 4 catalase activity in the whole blood and liver of both normal and diabetic group decreased when compared with the treated groups.

Table 5: Effect of Vitamin E on reduced glutathione in normal and diabetic rats

	Normal Control	50IU	100IU	200IU	400IU	Diabetic control	50IU	100IU	200IU	400IU
Blood	0.679±0.02	0.791±0.05	1.130±0.09 ^a	1.168±0.62	2.033±1.62	1.086±0.54	1.488±0.00	1.796±0.52 ^b	2.326±0.84	3.033±2.62
Liver	1.086±0.29	0.796±0.52	1.674±0.25 ^a	1.488±0.00	2.326±0.47	0.791±0.07	1.130±0.62	3.033±0.26	3.126±1.54 ^b	3.679±0.00

Values are Mean ± SD; Numbers with superscripts are significantly different (p<0.05) from control.

Treatment with vitamin E increased the level of blood GSH both in the normal and the diabetic treated rats but higher in the diabetic treated rats (Table 5).

Table 6: Effect of Vitamin E on lipid peroxidation in normal and diabetic rats

	Normal Control	50IU	100IU	200IU	400IU	Diabetic control	50IU	100IU	200IU	400IU
Blood (10)	2.208±0.00	1.909±0.00	1.971±0.00	1.505±0.62	0.876±0.00	7.099±0.00	6.054±0.00	5.681±1.51	3.504±1.21	0.876±0.00
Liver (10)	8.229±18.61	8.128±0.00	7.884±2.84	5.631±0.01	2.451±0.00	30.060±2.59	23.289±0.06	21.538±0.00	4.682±0.93	1.993±0.50

Values are Mean ± SD

As shown in Table 6, treatment of rats (normal and diabetic groups) with vitamin E caused a reduction in MDA level compared with the controls.

DISCUSSION

Free radical production is increased in subjects with non-insulin dependent diabetes mellitus (NIDDM) and those with essential hypertension but the mechanism responsible for the linkage among increased oxidative stress, impaired glucose metabolism and blood pressure is still debated. Vitamin E improves the free radical defense system potentially and may have a beneficial effect in improving glucose transport and insulin sensitivity (24).

Diabetes mellitus is characterized by elevated glycemia and frequently by concomitant glycosuria. The basic pathogenic agent producing hyperglycemia is missing or insufficient secretion of insulin or its insufficient action on the level of peripheral tissue cells. Metabolic consequences of this are not only in the metabolism of saccharides but quite often they are also manifested as disorder in the metabolism of proteins or as the disorder of metabolism of lipids, the pathogenesis of which is however more difficult (25).

The results obtained from this study have revealed that there is a relationship between vitamin E and glucose metabolism, as there was an increase in insulin sensitivity in diabetic rats treated with vitamin E. This was in agreement with the results of previous studies that antioxidant like vitamin E improves insulin sensitivity in diabetics (26). The reduction in the glucose levels of the diabetic control animals may also be due to partial recovery. Similar results were obtained by Kunkova *et al* (27) that partial spontaneous recovery of diabetes was associated with increased neuropeptide Y (NPY) levels in the atria. There was significant ($p < 0.05$) increase in the activity of SOD and reduced glutathione level. In a related study, erythrocyte SOD activity has been found unchanged, elevated or decreased in type 1 diabetic patients, whereas it was unchanged or lowered in type 2 diabetic subjects (28). Both SOD and catalase protect the cell from oxidative stress. SOD increases the formation of hydrogen peroxide which is converted to water in peroxisomes by catalase. It is therefore not surprising that generally an

increase in SOD is accompanied by a concomitant increase in catalase.

The insignificant decrease in the levels of the blood and liver glutathione content of the diabetic control compared with the treated groups show the importance of free radicals in causing oxidative stress, thus prevention using exogenous administration of vitamin E seems to be a logical alternative to the prevention of such injuries. Sailaja *et al* (29) reported that diabetic humans have shown increased lipid peroxidation and decreased level of glutathione, glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase.

Oxidative stress such as found in diabetes causes an increase in both plasma and liver Thiobarbituric Acid Reactive Substance (TBARS) as indicated in the samples' MDA content. In this study the high level of blood and liver MDA content in the diabetic control support the hypothesis that stress induced injury is mediated by lipid peroxidation process. This result also showed that vitamin E would reduce hemolysis by reducing the product of the lipid peroxidation process.

It can be concluded from the present study that diabetes mellitus itself induces lipid peroxidation and that administration of vitamin E prevents stress-induced elevation of lipid peroxidation. The protective effect of vitamin E is related to a decrease in lipid peroxidation and prevention of the reduction in blood and liver GSH content produced by the harmful effects of stress.

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